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Studies on the Nature of Physiologic Resistance to Puccinia Graminis Tritici

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STUDIES ON THE NATURE OF PHYSIOLOGIC RESISTANCE TO PUCCINIA GRAMINIS TRITICI[1]

WALTER N. EZEKIEL

INTRODUCTION

The cause of the resistance or the susceptibility of plants to the various Uredinales attacking them has been extensively investigated. Such investigations have been handicapped by the fact that rust fungi have not been successfully cultivated under artificial conditions; and for this reason research in this field in recent years has been limited largely to indirect, cytologic study of the processes involved.

The Uredinales include very highly specialized parasites. Within the species *Puccinia graminis tritici,* numerous physiologic forms have been discovered by Stakman and Levine (30). These forms are identified by their pathologic effect on varieties of *Triticum* spp. The differences by which these forms are distinguished are of a definite qualitative nature. The physiologic forms are classified merely by the type of infection produced. Thus, among the characteristics by which forms 18 and 19 are identified are differences in the reactions of Kanred wheat, C. I. No. 5146,[2] and Mindum, C. I. No. 5296. Kanred seedlings are susceptible to *P. graminis tritici* form 18, that is, however many or few rust pustules develop, there are large uredinia which often coalesce, and there are no hypersensitive areas on the leaves. Kanred seedlings inoculated with form 19, on the other hand, are immune or resistant; there may be no macroscopic evidence of infection at all or there may be hypersensitive or necrotic areas surrounding minute, isolated uredinia. Precisely the opposite is true of the variety Mindum, which is resistant to form 18 and susceptible to form 19. These are not the only differences between forms 18 and 19. Of the twelve wheat varieties used by Stakman and Levine to differentiate physiologic forms of *P. graminis tritici,* only seven react in ap-

[1] Report of investigations pursued under a National Research Fellowship in Botany at the University of Minnesota Agricultural Experiment Station, 1925-27. The writer takes pleasure in expressing his appreciation of the facilities made available by the Division of Plant Pathology and Botany and the Division of Agricultural Biochemistry; of the advice and encouragement of Dr. E. C. Stakman, Dr. R. A. Gortner, and virtually all the members of both divisions; and of the courtesy of E. C. Stakman, M. N. Levine, J. M. Wallace, H. E. Parson, R. U. Cotter, and S. J. Wellensiek in furnishing him with the identified physiologic forms of the various rusts with which they were working. He is particularly indebted for the use of unpublished data with regard to the hosts of new and undescribed physiologic forms, investigated co-operatively by the office of cereal crops and diseases of the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. This manuscript was submitted for publication in April, 1929.

[2] C. I. = Office of Cereal Investigations, United States Department of Agriculture, accession number.

proximately the same way to both forms. The complexity of the relation existing between the wheat plant and the parasite is evidenced by the fact that on the basis of inoculation tests on only twelve differential wheat varieties more than fifty physiologic forms within *P. graminis tritici* have already been discovered by Stakman, Levine, and their associates.

As was pointed out in a summary paper by Zimmermann (35), the relation is so complex that no single, common factor, such as the hydrogen-ion concentration of plants, for instance, could furnish an adequate explanation for physiologic resistance to the rusts. Physiologic resistance might, however, be the result of a complex series of simultaneous gradations in a number of common factors in the host plants, or of differences in specific materials, such as proteins, in different varieties and species. These differences might be assumed to be innate in the host tissues, or again, produced only following the stimulus of infection. The latter assumption, that vegetative growth of Uredinales is dependent on materials produced only in susceptible host cells and only after infection, would explain also the difficulty of growing rusts in artificial culture.

Whatever the immediate cause of physiologic resistance to rusts, it seemed to the writer that it should be possible to find some clue to it by studying the growth of different physiologic forms of rust fungi in extracts from plants susceptible or resistant to them. Such studies have been carried out with physiologic forms of *P. graminis tritici* and extracts of wheat varieties.

The investigations reported herewith were made with the dual aim of obtaining more direct information on the nature of resistance to rusts by a study of the growth of the parasites in extracts from host plants and on other substrata, and of attempting to develop methods of artificial culture applicable to Uredinales. Results relating to the first problem are considered in the present paper.

HISTORY

An elaborate summary of literature on the relation of Uredinales to their host plants is available in Zimmermann's monographic paper (35), and discussion with special reference to the cytologic results in papers by Allen (1, 2, 3, 4), Rice (25), and Wellensiek (34). Only papers of direct interest to the present work will be mentioned here.

Ward (33), Stakman (28), and others showed that the germ tubes of rust fungi may penetrate normally even into immune plants but usually die soon afterward. Resistance in this case thus appeared to be "physiologic"—the result of interaction of the host and the parasite—rather than the result of such morphologic differences as might

be concerned, for instance, in mechanical barring of infection by impenetrable epidermis or stomata. Hence the obvious experimental mode of attack on the problem of resistance was to grow rusts on extracts from susceptible and resistant plants and observe the differences; but this was apparently prohibited by the failure of efforts to grow Uredinales successfully in artificial culture. Few records of attempts in this direction are to be found in the literature, and the negative results of Ward (33) are doubtless typical also of other experiments that were unsuccessful and unrecorded.

Ward (33, p. 261) wished to "test the possibility of the leaf containing some body in the cell-sap which inhibits or promotes the growth of the fungus; and I did so by making a cold-water extract of the pounded fresh leaves, rapidly filtering through a stone or other filter, and sowing the spores in the liquid both raw and boiled. In all cases control sowings were made in water, and only those results regarded where the controls showed the spores to be vigorous.

"All attempts of this kind were in vain, however, since vigorous spores germinated equally well in extracts of the leaves of their own host-species and of their antagonistic host species. . . . The positive results do show, however, that the failure of spores from *B. mollis* . . . . to develop pustules on *B. sterilis*, for instance, is not due to a mere exudation of some antagonistic soluble extract—that the antagonism must be due to something far more subtle than a mere soluble poison oozing from the cells."

Leach (17) tried to determine the possibility of toxin or antitoxin formation following invasion. He germinated urediniospores of *P. graminis tritici* in extracts from normal and inoculated wheat seedlings, but was unable to correlate variations in the percentage of germination and the length of germ tubes with the resistance of the host from which the extract was made. He suggested, however, that rust resistance might be due to specific nutrient requirements of rusts, which would be satisfied only in congenial host plants.

Attempts have been made to correlate physicochemical properties of host plants with their resistance to rusts. Comes (7) suggested that the acidity of the cell sap was the property that enabled wheat plants to resist rusts. Miss Hurd, however, could not correlate the hydrogen-ion concentration of the expressed juice of normal wheat plants of different varieties with their relative susceptibility to stem rust (13), nor the changing acidity of different stages of wheat growth (14) with any variations in susceptibility to rust during the development of the plant.

Hursh (15) found that urediniospores of some physiologic forms of *P. graminis tritici* appeared to germinate at slightly different ranges of temperature and acidity. He was, however, unable to correlate

physicochemical properties of juice from normal wheat plants of different varieties with their resistance to rust (16).

Definite morphologic resistance has been shown to exist in the case of two stages of stem rust. Hursh (16) found that the mycelium of *P. graminis* is restricted in wheat stems to the collenchyma tissue, and thus explained the slight injury in varieties with little collenchyma tissue as due to this mechanical limitation of the spread of the mycelium. He emphasized, however, that this morphologic resistance was concerned only in determining the amount of damage that might occur in different varieties, and that "the differences in the reaction of wheat varieties to different biologic forms of *P. graminis tritici* appear to be due entirely to physiologic causes."

Melander and Craigie (21) have studied the nature of resistance of species of *Berberis* to *P. graminis*. They found that species with epidermis resistant to puncture were usually resistant to infection also. However, the reverse was not true in all cases, and they conclude that there probably is physiologic resistance also.

PRESENT INVESTIGATIONS

Material and Methods

All wheat varieties used were of the particular selections employed by Stakman and Levine (30) as differential hosts in the determination of physiologic forms of *P. graminis tritici*. Plants grown in the greenhouse were in five-inch pots of steam-sterilized soil. In the field, plants were drilled in rod rows as required. Greenhouse plants were grown on benches or in compartments that guarded against possible accidental rust infection, while field-grown material was exposed to natural infection.

In the preparation of extracts, plants were cut about one-half inch above the ground. The entire aerial portion was used, except noticeably dried or mechanically injured leaf tips, which were trimmed off. Grinding, when employed, was done in an ordinary meat grinder. The press cups used in the hydraulic press were of non-corrosive "Monel" metal. Small Berkefeld or Mandler diatomaceous filters of the coarser type (requiring only about five pounds of air pressure to force air bubbles through the thoroly wet filter cylinders) were used for ultrafiltration of plant extracts.

Materials to be used as substrata, including sterile distilled water, were stored in Pyrex tubes and flasks in the refrigerator. They were handled in sterile pipettes, with the usual aseptic precautions, in preparing dilutions from the concentrated materials.

Agar was the diluent for the plant extracts of some series. In preparing cultures in these series, the melted agar was cooled to 45°

C. before the required amount of extract was added, and held at this temperature while drops were removed.

Since observations were made of microscopic differences, hanging-drop cultures were employed almost exclusively. The substrata tested were therefore in contact only with cover-slips during the experiments. New cover-slips were used and cleaned as follows: autoclaved in cleaning solution, rinsed in distilled water, scrubbed with a camel's-hair brush under a stream of distilled water, rinsed and stored in alcohol. Glass rings and slides were also specially cleaned, stored in alcohol, and flamed off immediately before use. Rings were sealed to the slides with paraffin and to the coverslips with vaseline in the customary way. Hanging drops, of either liquid or melted agar substrata, were made with a needle loop of 4-mm. diameter; and this loop was not only sterilized but also washed between successive drops. No liquid was used at the bottoms of the cells.

Identified physiologic forms of the various rusts under investigation in the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station were available for inoculum. Most use was made of the forms of *P. graminis tritici* as identified by Stakman and Levine (30); and only a few cultures of *P. graminis avenae, P. graminis secalis, P. coronata,* and *P. sorghi* have been studied. Usually only one strain of a given physiologic form was studied. The accession numbers of the particular strains of *P. graminis tritici* used in this work are listed in Table IV.

Growth was studied from urediniospore inoculation only. Inoculum was taken from inoculated plants, usually grown in pots in the greenhouse, but occasionally in large culture tubes. In either case, inoculum to be used in a given series was collected from plants of approximately the same age and at the same period of rust development. Little Club wheat, C. I. No. 4066, is susceptible to almost all of the forms of wheat stem rust studied and was used to develop inoculum in most cases. *P. graminis tritici* forms 18 and 19, which were studied most extensively, were carried along on Little Club plants in neighboring wards in the greenhouse, separated only by a sheet of glass; and were periodically transferred at the same time to new plants, to furnish inoculum as uniform as possible.

For inoculation of cultures, urediniospores were taken from a number of pustules on different leaves and mixed in a dry, sterile Petri dish by stirring with a straight needle. A thin needle with a bent tip was run through this comparatively uniform mixture of spores, tapped lightly against the dish a definite number of times to remove most of the adherent spores and give a more regular number of spores per load, and then used to inoculate both of a pair of drop cultures. An inoculum of 50-150 spores per drop was customarily used except

when the density of inoculum was a variable of the experiment. In the case of liquid drops, the spores were distributed quite evenly throughout the drops by vigorous stirring with the needle. In agar drops, spores were distributed by touching the needle very lightly to the surface of the drop at a number of points in rapid succession, and the spores here were present in groups at some points in each drop and singly at other points.

During the whole investigation, all cultures were incubated in the dark at 20° C. or within about one degree of this temperature. Growth was usually complete in about 17 hours and measurements were made from 19 to 23 hours after inoculation. These were checked by final observations again in three days to a week, and the slight additional growth noted on rare occasions was added to the first figures. The results presented below are then the total growth obtained in various substrata under the conditions of the experiment rather than the growth within any specified time.

Observations were made on the living, unstained material. In most experiments the notes included counts of the number of spores in the drop, counts or estimates of the germination, codified notes on the general type of growth occurring, and measurements of 10 sporelings selected at random in different parts of the drop. As all cultures were run in duplicate, there were 20 sets of measurements for each dilution of each substratum tested. This appeared to be a fairly representative sample of the cultures, but further accuracy could have been obtained by additional replication. Growth was measured only in the case of spores sufficiently separated from others in the drop so that it could be seen that no fusion of adjacent sporelings had occurred; as when this happens the resulting growth may be much longer than that from a single spore. Measurements were made of the length of the main germ tube, and of the total length of all branches. Coiling, circumnutatory growth is frequent in urediniospore germ tubes and these measurements were partly estimates of the lengths of such curved portions. However, their reliability was indicated by the close agreement of successive sets of measurements in the same cultures. The same microscope and ocular micrometer were used throughout the investigation.

Characteristics of Physiologic Forms of Puccinia Spp. in Hanging-Drop Cultures

The varieties and physiologic forms of *P. graminis* were originally differentiated only by the reactions of the various host plants. Stakman and Levine (29, 18) have pointed out consistent differences in color, shape, and size of urediniospores of varieties of *P. graminis;* and Levine (19) demonstrated distinct differences between uredinio-

spores of physiologic forms within *P. graminis tritici*. Physiologic differences between these forms were noted by Hursh (15) in his studies of the relation of temperature and hydrogen-ion concentration to the germination of urediniospores.

In the work with hanging-drop cultures reported herein, the writer has found additional and readily demonstrable differences between the physiologic forms included. Growth was studied from about 200,000 urediniospores, and was measured for about 22,000 of the sporelings obtained. In very few of the cultures was the growth obtained much greater than that shown by Sappin-Trouffy (26), Plowright (23), and others. It was found that even this slight growth, extending usually less than 1000 μ and reaching its maximum development in less than 24 hours, showed definite, constant characteristics which differed not only for urediniospores of the same physiologic form on different media, but also for germ tubes of different physiologic forms grown under identical cultural conditions.

Fig. 1. Growth from Urediniospores of *P. graminis tritici* Physiologic Forms 18 and 19 A, form 19, and B, form 18; in sterile, distilled water, 25 hours. C, form 18 and D, form 19; in extract from normal Kanred plants, centrifuged and ultrafiltered (Table XXII) and diluted to 0.01 with sterile, distilled water, 2 days. Outlined with camera lucida, × 200.

Typical sporelings of form 18 and form 19, shown in Figure 1, A and B, illustrate differences characteristic of the growth of these forms in many substrata. The germ tubes of form 18 are longer than those of form 19, are coiling, and have few branches which, if present, are usually toward the tip. On the other hand, germ tubes of form 19 are shorter and rarely coiling, and have many branches which arise dichotomously at intervals along the entire germ tube and give it a scorpioid appearance.

Differences between germ tubes of the various physiologic forms are both qualitative and quantitative. The length of germ tubes, length of branches, and abundance of apical swellings are characteristics which were conveniently recorded quantitatively. These were considered fully in study of the results.

Other prominent differences are more of a descriptive nature and are not readily recorded quantitatively. For example, germ tubes may develop in a coiling or circumnutatory manner or remain quite straight; branching may be simple or dichotomous; and branches may be distributed along the germ tubes or localized near the tip or the base. Such differences have been observed, together with numerous gradations and combinations of them. Some of these differences are measurable, but summation or averaging of the measurements would hardly be valid and comparison of them would be difficult. These more qualitative differences have therefore been ignored in summarizing these studies.

Germination

Urediniospores of *P. graminis* have a number of germ pores, usually four, arranged equatorially. When the spores are placed in water, growth starts usually from two or more of the germ pores. There is apparently a strong "apical dominance" in the growth of the germ tubes, however, and only one germ tube develops to any considerable length.

In the laboratory the percentage of germination of urediniospores is frequently nearly 100 per cent in distilled water. The percentage of germination is not a good criterion for the comparison of substrata from this angle, as higher percentages would be impossible. The writer has found also that the percentage of germination may vary more with the age or methods of handling the urediniospores than do the characteristics of the germ tubes produced from these spores. In the present study the percentage of germination has accordingly been considered only in occasional experiments.

Length of Germ Tubes

As used in the present paper, "length of germ tube" is considered the length of the mycelium from the spore to the tip, or if a side branch was longer than that which seemed to be the main germ tube, to the tip of this branch. Length of germ tubes varies widely with changes in the environmental conditions, and, as shown below, even with the number of spores with which a drop is seeded. Variations according to environment are not necessarily the same for different physiologic forms of the same species. Thus, on one substratum germ tubes of a form may average distinctly longer than those of another, while on

a different substratum the relation may be just the reverse. Hence differences between physiologic forms based on lengths of germ tubes apply only for conditions specified.

This is demonstrated well in Table I with forms 18 and 19 of *P. graminis tritici.* In both sets of cultures on plain, one per cent agar, germ tubes of form 18 were nearly twice as long as those of form 19. In the dilute Shive's solution agar, however, germ tubes of form 19 invariably averaged longer than those of form 18. This relation between the growth of these two physiologic forms on plain agar and on Shive's agar was constant not only for the series listed in Table I but also in numerous experiments in which these physiologic forms were used repeatedly as checks in plain agar and Shive's agar.

TABLE I

EFFECT OF DIFFERENCES IN THE SOURCE OF INOCULUM AND OF CULTURE MEDIA ON THE CHARACTERISTICS OF PHYSIOLOGIC FORMS OF *P. graminis tritici* IN HANGING-DROP CULTURES, IN DUPLICATE AT 21° C.

Sources of inoculum	Substrata	Physiologic forms of *P. graminis tritici*	Mean lengths of germ tubes, in microns	Ratio, lengths of branches over lengths of germ tubes	Relative abundance of apical swellings†
Plants inoculated 36 days previously; pustules old	Plain 1% agar	18	310	0	+ +
		19	160	.174	?
	Dilute Shive's agar*	18	157	.109	+
		19	256	.147	?
Plants inoculated 15 days previously; pustules still fresh	Plain 1% agar	18	403	.008	+ +
		19	260	.120	+
	Dilute Shive's agar*	18	208	.098	?
		19	241	.206	—

* Shive's "best" solution (20) diluted to 0.1 concentration in one per cent agar.
† + + = numerous apical swellings; + = few; ? = doubtful; — = none.

Relation of density of inoculum to length of germ tubes.— All the various substrata tested, including the checks in sterile distilled water or plain agar, were run in duplicate for each physiologic form in each experiment. The average lengths of germ tubes produced in such duplicates were rarely closely similar. It was soon noted that germ tubes seemed to be longer in the drop that had been seeded with the greater number of urediniospores. Some variation between duplicates was to be expected, but the possibility that much of this variation might be due directly to differences in the density of inoculum made it desirable to study the relation of this factor to growth.

The results in distilled-water check cultures, from various experiments, were first considered. The relation between the number of spores per drop and the mean lengths of germ tubes obtained is shown in Figure 2 for such a collected series of cultures of form 18, and in Figure 3 for form 19. In both cases a definite positive correlation is evident between the number of spores present per drop and the mean

length of germ tubes produced. The correlation is $+ .49 \pm .09$ for form 19 and $+ .43 \pm .09$ for form 18. These correlations may be interpreted as indicating that with form 18 approximately 18 per cent, and with form 19 approximately 24 per cent, of the total variation in lengths of germ tubes shown in the graphs can be explained by differences in the density of inoculum.

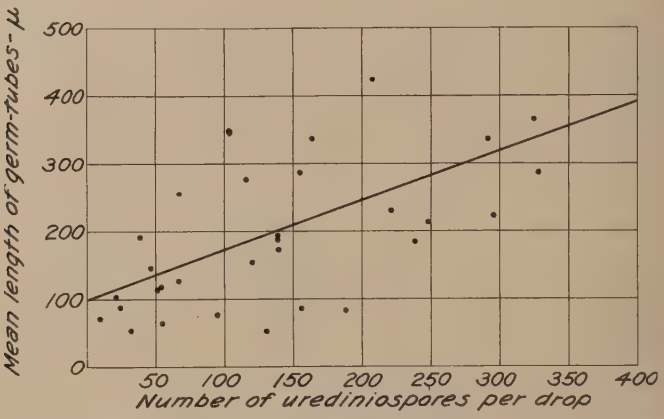

Fig. 2. Correlation of Number of Urediniospores of Physiologic Form 18, *P. graminis tritici*, per Drop of Sterile, Distilled Water, with the Mean Length of Germ Tubes Produced. $r = + .427 \pm .090$

It is to be remembered that the individuals of these series were not directly comparable, as they were merely check cultures picked out from series of cultures during a period of nearly three months. Variation owing to differences in the inoculum used (tho of the same strains of rust and from the same host plant, differing at times as to age of the pustule from which it was taken, seasonal differences in greenhouse conditions under which sporulation occurred, etc.), as well as possible slight differences in the cultural conditions, was to be expected. The true correlations here are doubtless considerably higher.

Data secured with other forms tended to confirm the validity of this relation. Some cultures of *P. graminis tritici* forms 1 and 14 were seeded at varying rates, all in hanging drops of Shive's "best" solution (20) diluted to 0.1 in distilled water and adjusted to pH 3.2 with N/100 HCl.

In 7 cultures of form 1, the mean lengths of germ tubes varied almost directly with the amount of inoculum (Fig. 4), from 45 μ in a culture with 15 spores to 597 μ in one with about 2,100 spores. The

correlation for the series was $+ .93 \pm .033$. With the 16 cultures of form 14 (Fig. 5) a mean length of germ tubes of 128 μ was produced in drops containing 50 urediniospores, while in a drop containing 670 spores the mean length was 420 μ. For this form the correlation was $+ .95 \pm .018$.

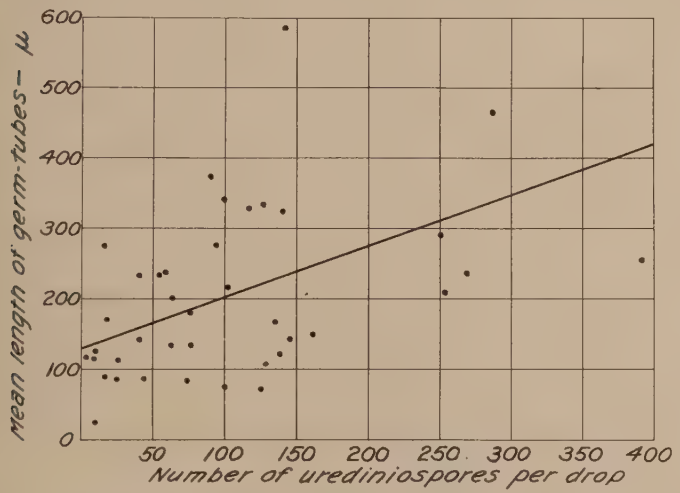

Fig. 3. Correlation of the Number of Urediniospores of Physiologic Form 19, *P. graminis tritici*, per Drop of Sterile, Distilled Water, with the Mean Length of Germ Tubes Produced. $r = + .492 \pm .090$

Whatever the cause of this increase of growth with increase of density of inoculum, the fact that it does occur appears unquestionable. Also, since it is impracticable to set up extensive experiments of this kind with a very carefully controlled number of spores in each drop, results of growth in various substrata should be corrected for this factor before they are strictly comparable, if small differences are to be significant. Such a correction has been used at times with forms 18 and 19 on the basis of the data shown in Figures 2 and 3. The regression formulae calculated from these figures were possibly better for this purpose because of the fact that the data concerned were composites of many series; and the mean check values calculated were perhaps more truly representative than if they had been based on the results of single series.

Values were calculated along the regression lines for cultures of forms 18 and 19, respectively, at each change of five spores per drop. This furnished estimated "check" cultures with densities of inoculum

corresponding to those of any cultures that might be considered. Such estimated check values were usually subtracted from the average length of germ tubes obtained experimentally in the media tested, thus eliminating density of inoculum as a variable factor. The results were considered "net increases," either positive or negative, in length of germ tubes for the particular substrata used in the experiment as compared to growth in sterile, distilled water. As a matter of fact,

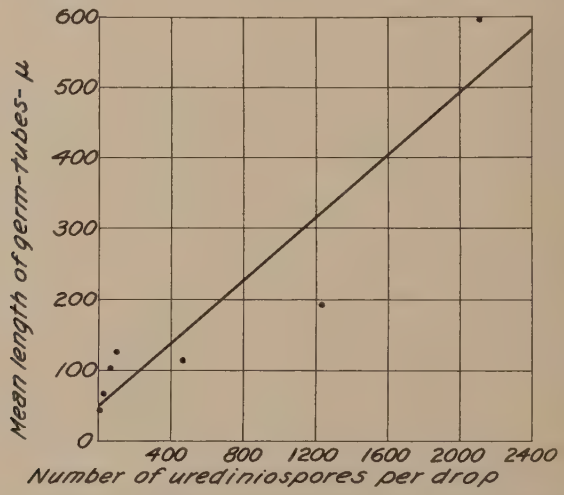

Fig. 4. Correlation of the Number of Urediniospores of *P. graminis tritici* Physiologic Form 1, per Drop of Diluted Shive's Solution, with the Mean Length of Germ Tubes Produced. $r = +.933 \pm .033$

however, this interpretation is not always quite correct. It presupposes that the rate of increase with density of inoculum will be the same in each substratum tested as it is in water, which is probably not true. This rate appears to be lower in most substrata than it is in distilled water.

To minimize error, therefore, the amount of inoculum has been kept as nearly constant as possible, even tho a correction for density of inoculum could be applied. In any case, use of the estimated check values has furnished a method of showing that observed results in tests of substrata are probably due to the factors under examination rather than to variations in density of inoculum. Application of the correction formulae to substrata other than distilled water probably over-corrects for differences in density of inoculum; and experimental results would appear unimpeachable from this angle when the same rela-

tion is shown, whether or not the estimated check values have been used.

Density of inoculum and length of germ tubes in agar drops.—The correlations shown above were of growth in liquid substrata. Many other series were run in which the nutrients were diluted in one per cent agar. While the density of inoculum did not appear so important here as in liquid media, it seemed desirable to ascertain whether it needed to be considered as a factor.

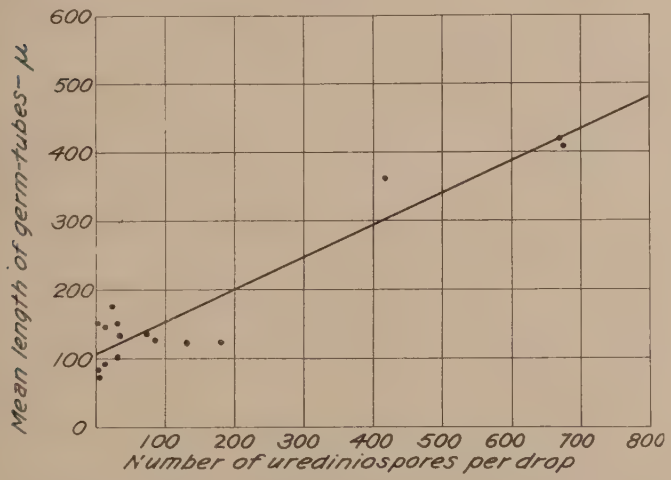

Fig. 5. Correlation of the Number of Urediniospores of *P. graminis tritici* Physiologic Form 14, per Drop of Diluted Shive's Solution, with the Mean Length of Germ Tubes Produced. $r = +.945 \pm .018$

Forms 1, 18, and 19 of *P. graminis tritici* were seeded at varying rates in hanging drops of plain, one per cent agar. The results confirmed those previously obtained. The length of the germ tubes of each form increased with increased density of inoculum (Fig. 6). On the agar, however, growth was relatively greater at the lower concentrations of inoculum than in liquid substrata. In the latter, the regression lines, showing the best estimated values of lengths of germ tubes on the basis of density of inoculum, tend to approach zero as the density of inoculum decreases. In the agar cultures, on the contrary, with even fewer than 10 spores in a drop, the germ tubes usually averaged more than 200 μ in length.

Further correlations of growth with density of inoculum have been made, using assembled data of the growth in plain, one per cent agar drop cultures that were run from time to time as checks for different

series. The results, along with the three correlations discussed above, are given in Table II.

Decided positive correlation may be noted in the case of each rust form used. The coefficients of correlation, as might be expected, are higher in series in which there were fewer cultures. The regression equations in the last column of Table II give the method by which

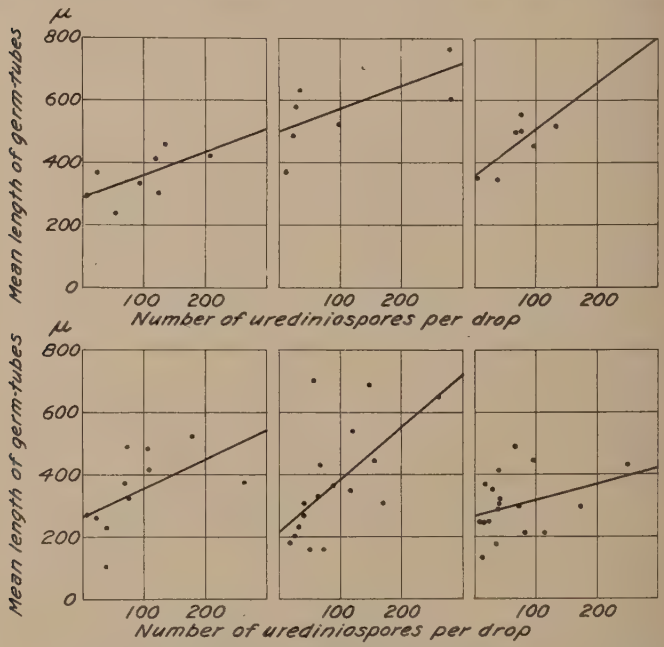

Fig. 6. Correlation of the Number of Urediniospores per Drop of Plain One Per Cent Agar with the Mean Length of Germ Tubes Produced

Left to right, physiologic forms 1, 18, and 19. Upper row based on results from single series, lower row on assembled data from several series. Coefficients of correlation in Table II.

probable values can be assigned to lengths of germ tubes on the basis of the number of spores per drop. In these equations the constants by which "A" (the number of spores) is multiplied express the rate at which increase in number of spores increases length of germ tubes; while the second constant of each equation is the estimated length of germ tubes with "0" spores per drop, and is added to the first product to obtain the actual length in microns. Both of these constants varied with the different forms. There appears little reason to doubt that variation of this sort actually exists between physiologic forms of *P.*

TABLE II

SUMMARY OF CORRELATIONS OF THE NUMBER OF UREDINIOSPORES PER DROP AND THE MEAN LENGTH OF GERM TUBES PRODUCED, IN ONE PER CENT PLAIN AGAR HANGING-DROP CULTURES

Physiologic forms of *P. graminis tritici*	No. of cultures per series	Coefficients of correlation	Regression equations*	
1†	11	+ .547 ± .142	X = .922A + 268	.825A + 277
1‡	8	+ .646 ± .139	X = .728A + 285	
9†	8	+ .691 ± .124	X = .895A + 230	
17†	12	+ .569 ± .132	X = .456A + 310	
18†	17	+ .598 ± .105	X = 1.68 A + 220	1.201A + 360
18‡	8	+ .658 ± .135	X = .722A + 500	
19†	18	+ .339 ± .141	X = .523A + 272	1.024A + 312
19‡	7	+ .745 ± .113	X = 1.525A + 352	
27†	8	+ .687 ± .126	X = .382A + 217	
Mean regression value, used later in correction of experimental results for variations in density of inoculum			X = .87A +	

* X = estimated lengths of germ tubes, in μ; A = number of spores.
† Assembled data from several series.
‡ Data from a single series.

graminis tritici. It is quite probable, for instance, that both the rate of increase and the characteristic constant to be added are higher for form 18 than for form 19.

On the other hand, equations calculated from single series of cultures grown simultaneously are quite different for each form from those calculated from assembled data of many series. Such variation might be encountered in any series to which these equations might be applied; therefore it has seemed needless to try to use individual equations for the different forms. The equation averaged from those in Table II has been used instead, in correcting results, with any form of *P. graminis tritici*, for differences in density of inoculum in agar drops in the experiments to be considered below.

The results given in Figures 2 to 6 and Table II show that in drop cultures inoculated with urediniospores of *P. graminis tritici* the mean length of germ tubes increases with the density of inoculum. The cause is not yet known, but the following explanation appears probable. Urediniospores contain stored materials which migrate into the germ tubes as growth starts. A portion of these materials may diffuse more or less slowly out of the germ tube into the surrounding substratum. Then, the greater the number of germ tubes in a given drop, the higher will become the concentration of soluble nutrient or stimulating materials in the drop available for absorption by germ tubes. The difference between results in liquid and agar drops is also explained by this hypothesis. Diffusion away from germ tubes in agar would be much slower than in liquid drops; hence, even at the lowest concentration of inoculum, we should expect the relatively long germ

tubes which were actually found. Such variation according to density of inoculum as does occur in the agar cultures is probably the result of diffusion of materials in a surface film of water, rather than of diffusion through the agar. While it is probably the area of mycelium present per drop that affects the length attained per germ tube, what was counted (as a matter of convenience) was the total number of spores present. The correlations above might be still higher were they calculated instead from the number of spores that germinated.

Length of Branches and Branching Ratios

The total length of branches of the germ tubes was secured simply by summation of the length of all branches. There is considerable variation in the extent of branching of different physiologic forms, even of the same species. This is shown not only in the length of the branches, but also in the number of branches per germ tube. The number and size of branches is, of course, affected by cultural conditions also.

In comparing branching in different substrata or by different forms it has been convenient to correct for the differences in extent of growth in different media by using a "branching ratio," obtained by dividing the average total length of branches per germ tube by the average length of germ tubes. This ratio is larger than the portion of the total vegetative growth of the young sporeling that consists of branches. Thus, with a germ tube measuring 200 μ and branches totaling the same length, only half of the total growth would consist of branches. The branching ratio (200 divided by 200) would, however, be 1.0. Furthermore, these ratios are based on the extent of branching only, without regard to the number of branches per germ tube that may be included to make up the total length of branches.

Differences in branching ratios of different physiologic forms are relatively constant and have been of value in the comparison of substrata.

Apical Swellings on Germ Tubes

It has been observed previously that the dense spore contents of germinating urediniospores migrate out into the germ tube (Fig. 7, A) and toward its apex. Not infrequently these materials reach the tip of the germ tube, which may then become more or less inflated and appear somewhat sporelike. Such inflations of the tips of rust germ tubes were described by Tulasne (31, p. 152) as early as 1854, and have been noted since by Plowright (23), Büsgen (5), Hitchcock and Carleton (12), Sappin-Trouffy (26), and Spaulding (27). They have been considered generally merely as anomalous growths due to the abnormal conditions of artificial culture.

Apical swellings have been produced, apparently normally, by all the physiologic forms of all species studied during this investigation, tho not necessarily at the same rate in each case. The bodies have been produced on a wide variety of substrata: distilled water, plain agar,

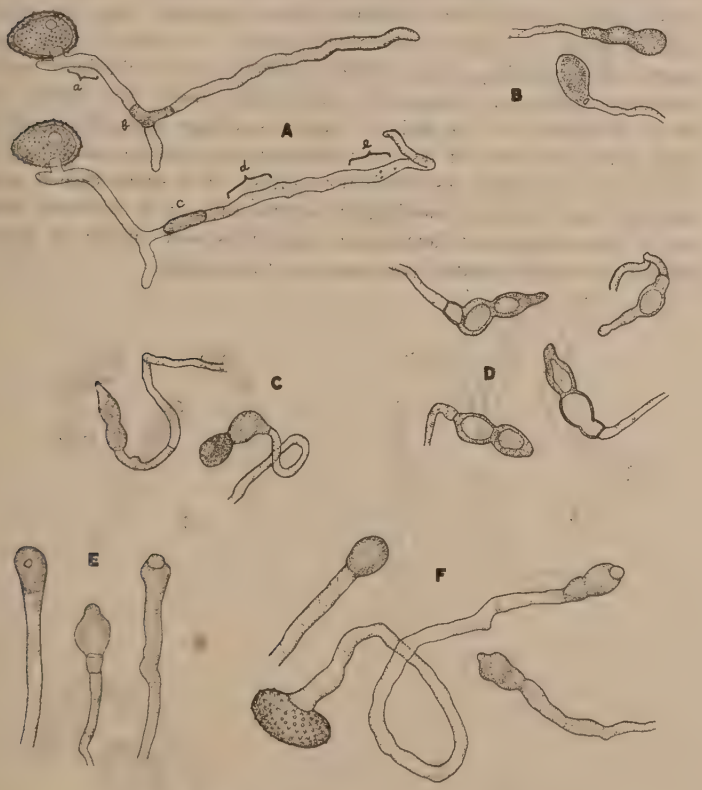

Fig. 7. A, progress of spore contents toward tip of a germ tube of form 1, in an agar hanging-drop culture at room temperature of 21° C. Time since inoculation, *a*, 3 hours; *b*, 3 hours, 45 minutes; *c*, 4 hours; *d*, 5 hours; and *e*, 23 hours.

B to F, apical swellings on tips of germ tubes of *P. graminis tritici* in hanging-drop cultures. B, D, and E, form 1; C, form 14; and F, form 23. Substratum plain one per cent agar, except for D which was in extract from normal Kanred plants diluted in agar to .01. (Table XXVII.) Camera lucida, × 400.

Shive's solution, M 1000 dextrose solution, wheat extracts, autolyzed yeast decoctions, etc. They have been seen on sporelings quite isolated from any other growth in the cultures, so that fusion of colonies is not a prerequisite for their production.

Series of hanging-drop cultures of various physiologic forms of *P. graminis tritici, P. graminis avenae, P. graminis secalis,* and *P. coronata avenae* were grown on plain agar for the express purpose of studying the apical bodies. They were found in cultures of all the rusts mentioned. The swellings usually were spheric to clavate, with dense, brown contents but without definite septation from the germ tube, as shown in Figure 7, F, and Figure 8, A, B, and C. Less frequently these swellings were divided from the germ tube by a well-developed wall, and occasionally the swelling itself was septate as shown in Figure 7, C, D, and E. Typical swellings when first formed were nearly the color of normal urediniospores, and the most highly developed very closely resembled teliospores. In a single case it was observed that the apical swelling on a germ tube of *P. coronata* bore on its tip a number of denticulate outgrowths very similar to those present on normal, mature teliospores of this species.

Fig. 8. Apical Swellings in Hanging-Drop Cultures in Plain One Per Cent Agar
A, *P. graminis avenae* form 4 (Acc. No. 8406); B, *P. graminis secalis* form 11 (Acc. No. 1317); C, *P. graminis secalis* (Acc. No. 1374); and D, *P. coronata avenae* (H. E. Parson's Acc. No. 6). Camera lucida, × 400.

No further development of these bodies has been observed. At least in the hanging-drop cultures studied, they appear to reach their fullest development within 24 hours, tho additional septation apparently may occur (or possibly merely becomes more noticeable) up to 36 or 48 hours, after which, gradual degeneration usually starts. The contents of the inflated tip lose the brown color derived from the translocated urediniospore material and become first olivaceous or greenish brown, then densely protoplasmic but hyaline, and later more and more vacuolate. (See Fig. 9, B.)

Fig. 9. A, unusual type of degeneration of apical swelling, *P. coronata avenae* (H. E. Parson's Acc. No. 6).

B, usual slow degeneration of apical swellings. *P. graminis tritici* form 29, culture on plain one per cent agar, drawn 15 days after inoculation. Note vacuolation even of the tips of the germ tubes. Outlined with camera lucida, × 400.

Apical swellings on germ tubes of *P. coronata* generally were of a less clearly defined shape than those of *P. graminis tritici*. Those shown in Figure 8, D, are quite representative of certain series. An unusual type of disintegration observed in one case with *P. coronata* is shown in Figure 9, A. This germ tube was in a hanging-drop culture on plain agar, incubated for 17 hours at 21° C. The culture was taken out of the incubator for microscopic examination at room temperature, about 26° C. Just as the swelling shown in Figure 9, A, had been noted, about 20 minutes after the slide was removed from the incubator, the contents apparently exploded the side of the inflated tip. The oily droplets that exuded were brown.

As mentioned above, apical swellings are not produced in the same abundance by different physiologic forms of the same species. For example, they were produced on a much higher percentage of germ tubes of form 18 than of form 19 of *P. graminis tritici*, and this relation held true through many series during a period of two years. Similarly, some physiologic forms of *P. graminis tritici*, such as forms 1 and 23, produced these bodies in profusion, frequently on nearly

every germ tube in a series, while other forms produced very few. The rate of production seemed roughly proportional to the abundance of teliospore production by these forms on plants of Little Club wheat in the greenhouse. The production of teliospores by 8 physiologic forms of *P. graminis tritici* on Little Club wheat seedlings that were grown in the greenhouse in large glass tubes on cotton moistened with Shive's solution is recorded in Table III. Notes on tubes in which plants had been inoculated one, two, and three months previously are included; but unfortunately there were only a few plants infected with each physiologic form. In general, the physiologic forms that produced large numbers of apical swellings in artificial culture also produced teliospores abundantly on Little Club plants, which are susceptible to all of the forms included in Table III. There would be much closer correlation here except for the few apical swellings produced by form 27. The apparently aberrant relation of this and other physiologic forms to which Vernal emmer is susceptible will be discussed in some detail later.

TABLE III.

Comparison of Abundance of Teliospores Produced on Little Club Wheat Seedlings, Grown in Glass Tubes, with the Abundance of Apical Swellings Produced by the Same Physiologic Forms of *P. graminis tritici* in Plain-Agar Hanging-drop Cultures

Physiologic forms of *P. graminis tritici*	Teliospores on Little Club wheat plants	Average percentage of apical swellings on germ tubes in artificial cultures
14	None	5
19	do	6
21	Occasional	29
1	do	47
18	do	22
15*	do	16*
23	Profuse	49
27*	do	11*

* Forms 15 and 27, to which Vernal emmer is susceptible, are aberrant in most classifications of these results.

The evidence at hand on the morphologic resemblance of apical swellings to the teliospore stage of rusts, and on the somewhat low correlation of the rate at which they are produced with the abundance of normal teliospore production on wheat plants, is perhaps insufficient to prove that apical swellings on urediniospore germ tubes are actually immature teliospores. The writer would suggest that this is probably the case, altho conclusive proof is still lacking.

Relation of density of inoculum to production of apical swellings.—Altho the length of germ tubes was affected to a marked degree, both in liquid and agar drops, by the numbers of spores present per drop, there did not appear to be so pronounced an effect of density

of inoculum with regard to the percentage of germ tubes that produced apical swellings. In general, the relation seemed to be that the fewer the spores per drop, the more abundant the apical swellings.

Data are given in Figure 10 for the production of apical swellings and the number of spores per drop in a collected series of cultures of *P. graminis tritici* form 1 in plain agar. The correlation here is low ($-.253 \pm .145$) but still plainly negative.

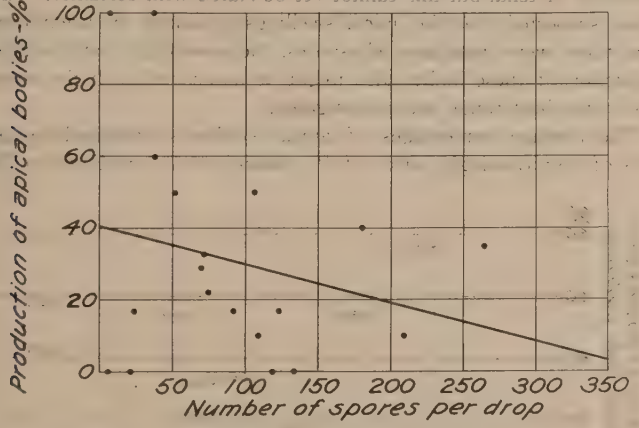

Fig. 10. Correlation of the Percentages of Germ Tubes Producing Apical Swellings, in Plain-Agar Drop Cultures of *P. graminis tritici* Form 1, with the Numbers of Spores per Drop. $r = -.253 \pm .145$

Corrections in results of experiments in which the amount of inoculum varied were occasionally made on the basis of the regression line shown in Figure 10, thus using the regression equation with form 1 as a general one to apply to all physiologic forms of *P. graminis tritici* on plain agar. This usage is not quantitatively correct but has probably not introduced any important error into the results.

The relation here was to be expected, in that increased density of inoculum had an effect on the production of apical swellings opposite to that found for the length of the germ tube. Germ tube elongation rather clearly represents vegetative growth, while the apical swelling appears to indicate a tendency toward reproduction, even if abortive. It is possible that germinating urediniospores, almost irrespective of environment, tend to enter a resting stage quickly, and transfer their contents into teliospores, unless some rather specific conditions inhibit reproduction and permit vegetative development.

Relation of production of apical swellings to the host range of physiologic forms.—There were marked differences between

physiologic forms with respect to the percentage of germ tubes that produced apical swellings. The question arose as to the physiologic significance, if any, of these differences.

Parson (22) had noted considerable differences in the readiness with which physiologic forms of *P. coronata avenae* produced telia on host plants, and concluded that "it seems likely that there is a correlation between the narrow pathogenic specialization of a strain and early formation of telia, but this cannot yet be stated with certainty." Since it was suspected that the apical swellings produced on urediniospore germ tubes were immature teliospores, the possibility that the rate of production of these bodies also might be associated with the narrowness of the host range was investigated.

The average percentages of apical swellings produced by germ tubes of 19 physiologic forms of *P. graminis tritici* are listed in Table

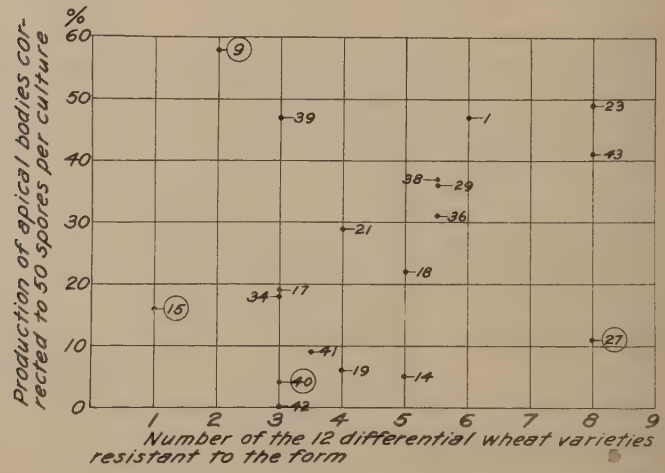

Fig. 11. Relation of the Mean Production of Apical Swellings by Physiologic Forms of *P. graminis tritici* in Plain One Per Cent Agar Hanging-Drop Cultures, to the Host Ranges of These Forms

Forms to which Vernal emmer is susceptible are shown in circles. Omitting these forms and form 39, $r = +.791 \pm .067$.

IV and shown graphically in Figure 11. The percentages are fairly accurate; enough cultures of most physiologic forms were grown so that the average percentages were changed only slightly when results from individual cultures were corrected for variations in density of inoculum. There were 7 physiologic forms to which more than 5 of the 12 wheat varieties are resistant. Apical swellings were produced on more than 30 per cent of the germ tubes of all but one of these

forms. On the other hand, there were 12 physiologic forms to which 5 or fewer wheat varieties are resistant. Apical swellings were produced on less than 30 per cent of the germ tubes of all but two of these forms. In other words, physiologic forms of *P. graminis tritici* with relatively restricted host ranges usually produced more apical bodies than those forms with wider host ranges.

TABLE IV

PERCENTAGE OF APICAL SWELLINGS ON UREDINIOSPORE GERM TUBES OF PHYSIOLOGIC FORMS OF *P. graminis tritici* IN PLAIN, ONE PER CENT AGAR HANGING-DROP CULTURES, AND HOST RANGES OF THE FORMS

Physiologic forms of *P. graminis tritici**	Host range. No. of the 12 differential wheat varieties resistant to the form	Percentage of germ tubes producing apical swellings, corrected to 50 spores per drop
1 (Acc. No. 2)	6	47
9 (Acc. No. 1576)	2	58
14 (Acc. No. 581)	5	5
15 (Acc. No. 849)	1	16
17 (Acc. No. 1312)	3	19
18 (Acc. No. 957)	5	22
19 (Acc. No. 1297)	4	6
21 (Acc. No. 1497)	4	29
23 (Acc. No. 1281)	8	49
27 (Acc. No. 846-c)	8	11
29 (Acc. No. 738)	5.5†	36
34 (Acc. No. 1575)	3	18
36 (Acc. No. 1097)	5.5†	31
38 (Acc. No. 794-b)	5.5†	37
39 (Acc. No. 1163)	3	46
39 (Acc. No. 1605)	3	48
40 (Acc. No. 1010-e 2)	3	4
41 (Acc. No. 1010-f)	3.5†	9
42 (Acc. No. 1010-i)	3	0
43 (Acc. No. 1569)	8	41

* Numbers in parentheses are University of Minnesota, Division of Plant Pathology and Botany accession numbers.

† Varieties in which the heterogeneous or "X" type of infection occurred were counted as half resistant.

This relation is shown also by correlations between the number of varieties resistant to physiologic forms and the percentages of apical swellings produced by them. Including all forms shown in Table IV and Figure 11, $r = +.265 \pm .144$.

The discrepancies in these results are of special importance in that they occurred also in some other series to be discussed below. It is evident that forms 9, 27, and 39 constitute exceptions to the general results. Considering only the other forms in Figure 11, it would appear that form 27 produced fewer apical swellings than would be expected while forms 9 and 39 produced many more. These aberrant forms were tested repeatedly, and the results confirmed the values shown. Two different isolations of form 39 have been tested (Table IV), with almost identical results. The validity of the apparently aberrant figures appears unquestionable.

Considering again only the general course of the results, form 15 appears also to lie at a definite distance above the expected curve. It is to be noted further that Vernal emmer is resistant to almost all of the identified physiologic forms of *P. graminis tritici.* Of the first 43 forms studied by Stakman, Levine, and their associates, there are only 6 to which this variety is susceptible. The 4 forms included in these studies are indicated in Figure 11 by circles around the form numbers. Of these 4 forms, 3 were aberrant to the general course of the results. Whether this relation is other than fortuitous remains to be determined and will be discussed further below; but for the present we may assume that those forms that attack Vernal emmer successfully have some common characteristic which differentiates them from most of the other forms of *P. graminis tritici.* This common characteristic so affects the growth of these forms in plain agar drop cultures that the production of apical swellings is not indicative of the host ranges of the forms. If we omit from the calculation those forms to which Vernal emmer is susceptible, the correlation becomes much higher, $r = + .597 \pm .112$. If we further omit form 39, tho without explaining why it should be grouped with the "Vernal emmer group," the correlation becomes appreciably higher again, $r = + .791 \pm .067$.

These results indicate strongly that in *P. graminis tritici* the physiologic forms that are weaker pathogenically, if that term may be used in speaking of forms with relatively more restricted host ranges, produce high percentages of apical swellings on uredinial germ tubes in plain, one per cent agar; while forms with wide host ranges produce fewer of these apical, teliospore-like bodies. A definite group of physiologic forms, including form 39 and those tested to which Vernal emmer is susceptible, do not fit this general correlation. In view also of further results with these forms, it appears probable that they differ in some fundamental physiologic relation from the many others that have been studied. Whether this difference involves factors that are concerned also in parasitism, or is merely a peculiarity of response to cultural conditions that masks the usual correlation, cannot be stated from the results at hand.

Puccinia graminis tritici in Extracts from Host Plants

The concept of the physiologic nature of resistance to rusts has rested to a certain extent on negative evidence. Varietal resistance to the different physiologic forms of *P. graminis tritici* could not be explained in other ways, as for instance, by morphologic characteristics of the host; the hypothesis that resistance is owing at least in part to differences in the value of different plants as substrata for the rusts remained as a possible explanation. The significant differences were

variously assumed to be materials present or produced in resistant plants, but absent in other plants, which inhibited growth of the parasite (33); or materials present only in susceptible plants, which furnished nutrients necessary to the development of the fungus (17).

Direct attack on the problem was attempted in the present work by culturing urediniospores of various physiologic forms of *P. graminis tritici* in extracts from different wheat varieties. Introductory series were run first, mostly with extracts expressed after freezing the tissues. The results appeared of some value, but were finally considered inconclusive as the methods used were in process of development and each successive refinement tended to throw doubt on the validity of earlier data. Thus, it was found that anastomosis of adjacent sporelings resulted in longer growth than that secured from a single spore, so that for length of germ tube measurements it was necessary to select spores well separated in the drop cultures. The discovery that the length of germ tubes is influenced by the density of inoculum, made it desirable to count the number of spores in each drop, for accurate comparison.

The preliminary series were, however, of some value in addition to that of affording the opportunity of perfecting the technique. They showed that germination counts were much more variable, within presumably similar conditions, than growth measurements. In view also of the fact that germination of rust spores occurs readily in distilled water, and apparently with little regard to host relations, germination counts were not included in the later experiments.

It was found that wheat extracts required considerable dilution before urediniospores could germinate and growth occur (illustrated also in Tables VI, VIII-XII, XVI, and XVII). Growth was often poorer in extracts diluted to .05 than in the checks; while in further dilutions to .005 it was considerably better than in check cultures. Minute proportions of host-plant extracts were thus sufficient to influence the growth of the rust.

The following experiments were performed with the improved methods described and have furnished direct evidence that there is a physiologic basis for resistance to Uredinales.

Experiment I

Methods: Kanred and Minduni wheat were sown in rows in the field, August 17, 1926. The plants were exposed to natural infection, but were attacked to a very slight extent, if at all, before harvest. The dates of harvest and methods of extraction used for the different lots are given in Table V. It is to be noted that the extracts in lots 2, 6, 7, and 8 were not diluted during preparation, while the decoctions of

lots 3, 4, 5, and 9 were necessarily in water; and that these differences in original concentration of the plant extracts were disregarded in the further dilution for culture substrata. All materials were used as prepared, and in further dilutions with sterile, distilled water to .25, 0.1, .01, and .001, as substrata for hanging-drop cultures of physiologic forms 18 and 19 of *P. graminis tritici*. Duplicate hanging-drop cultures were inoculated with urediniospores of each physiologic form with each dilution of each extract or decoction. The two wheat varieties react reciprocally to these physiologic forms. Kanred is susceptible to form 18 and practically immune from form 19, while Mindum is resistant to form 18 and susceptible to form 19.

TABLE V

PROCEDURES USED IN EXPERIMENT 1 FOR THE PREPARATION OF EXTRACTS FROM KANRED AND MINDUM SEEDLINGS

Lot	Material	Preparation	Sterilization
2*	Mindum plants, cut September 25	Ground; pressed in Monel metal cups by direct hand pressure only	Filtered aseptically through sterile filter paper
3	200 gm. plants, cut September 28	Ground; steamed in 500 cc. distilled water in Arnold sterilizer, 2 hours; filtered through cheese-cloth and filter paper	Autoclaved 15 minutes at 15 pounds pressure
4	200 gm. plants, cut September 29	Ground; steeped in 500 cc. cold, distilled water, at 22° C., 4½ hours; strained through cheese-cloth	Ultrafiltered through Berkefeld filter
5	200 gm. plants cut September 29	Ground; steeped in 500 cc. cold, distilled water, at 22° C., 4½ hours; strained through double cheese-cloth and double filter paper	Autoclaved 15 minutes at 15 pounds pressure
6	500 gm. plants, cut October 10	Ground; pressed at 0 pressure (hand pressure only); filtered through filter paper	Ultrafiltered through Berkefeld filter
7	(Material left in press from lot 6)	Pressed at 0-50 kgm. per cm.² in hydraulic press; filtered through filter paper	Ultrafiltered through Berkefeld filter
8	(Material left in press from lot 7)	Pressed at 50-150 kgm. per cm.² in hydraulic press; filtered through filter paper	Ultrafiltered through Berkefeld filter
9	(Material left in press from lot 8)	Stored two days in refrigerator; heated in 500 cc. distilled water in autoclave, 15 minutes at 15 pounds; decanted; filtered hot through filter paper	Autoclaved 15 minutes at 15 pounds pressure

* Mindum only in this lot; all others apply to Kanred and Mindum.

Length of germ tube results.—The length of germ tube measurements of these series have been studied in some detail (Tables VI to XIII). As shown above, germ tubes of form 18 sporelings are

longer than those of form 19 when grown in purely synthetic media and under precisely similar conditions. Direct comparison of the growth in plant extracts would therefore be misleading and difficult to interpret. We might obviate this difficulty by comparing the magnitudes of differences observed here between the two forms with those observed between the checks—cultures in distilled water—in each series. However, it was also shown above that the lengths of germ tubes in drops of sterile, distilled water, as of other media, are greatly affected by the numbers of urediniospores used in inoculating the drops. The following proved an expeditious way of correcting simultaneously for both of these factors.

TABLE VI

NET INCREASES IN LENGTHS OF GERM TUBES OF *P. graminis tritici* FORMS 18 AND 19 IN EXTRACTS FROM KANRED AND MINDUM WHEAT, DILUTED IN STERILE, DISTILLED WATER*

Lot No.	Wheat extracts	Physiologic forms of *P. graminis tritici*	Net increases in lengths of germ tubes, in microns, in extracts diluted to concentrations indicated					
			Mean, all concentrations	1.0	.25	.1	.01	.001
2	Mindum	18	48	....	....	162	—30	12
		19	299	....	363	498	196	138
3	Kanred	18	36	....	—58	46	48	106
		19	132	....	....	107	162	128
	Mindum	18	41	—70	—49	218	94	10
		19	38	....	112	—53	160	—68
4	Kanred	18	230	....	63	442	394	22
		19	189	....	171	345	95	147
	Mindum	18	3	—10	60	24	—61	....
		19	96	....	164	177	73	—31
5	Kanred	18	86	—99	280	97	114	39
		19	1	—58	—101	68	114	—17
	Mindum	18	70	31	266	—5	10	49
		19	31	—90	157	15	102	—31
6	Kanred	18	287	....	191	176	324	457
		19	147	....	142	162	193	92
	Mindum	18	0	....	....	—80	82	—2
		19	53	....	....	—191	12	339
7	Kanred	18	154	....	38	162	70	346
		19	105	....	72	242	76	29
	Mindum	18	39	....	53	—38	17	123
		19	82	....	....	—43	193	97
8	Kanred	18	—59	....	....	....	—20	—99
		19	—56	....	....	—92	19	—94
	Mindum	18	175	....	235	....	287	2
		19	89	....	....	31	143	94
9	Kanred	18	—79	—234	51	2	—147	—65
		19	0.5	....	41	46	—128	43
	Mindum	18	—112	—141	—107	—104	—15	—191
		19	3	....	—9	—27	25	22

* Recorded lengths, minus (distilled water) checks estimated for the actual densities of inoculum of the individual cultures, minus the mean divergence of actual checks of particular series from estimated values for these checks.

TABLE VII

NET INCREASES IN LENGTHS OF GERM TUBES ON KANRED AND MINDUM SUBSTRATA, AS COMPARED TO ESTIMATED GROWTH IN STERILE, DISTILLED WATER, EXPRESSED AS MEAN VALUES, INCLUDING ALL CULTURES IN THE SERIES AND DISREGARDING THE VARYING DILUTIONS OF SUBSTRATA

Wheat extracts	Physiologic forms of *P. graminis tritici*	Means of increases in lengths of germ tubes, in microns					
		All lots		Lots 2, 4, 6, 7, and 8 (not heated during preparation)		Lots 3, 5, and 9 (heated during preparation)	
		(a)*	(b)*	(a)	(b)	(a)	(b)
Kanred	18	121	98	197	183	9	13
	19	86	72	86	107	74	38
Mindum	18	8	26	45	49	46	0.3
	19	39	86	86	133	34	24

* (a) Increases estimated from checks calculated by formula only, that is, corrected for variations in density of inoculum but not for differences between series.

(b) Corrected also by divergence of the actual checks of the individual series from estimate (as in Table VI) so that results are completely comparable.

Tables of estimated length of germ tube values for different numbers of spores per drop of distilled water were calculated from the data of Figures 2 and 3 for the two forms. From these tables "estimated check values," which represented growth in distilled water drops containing the same numbers of spores as each hanging-drop culture under consideration, were selected for each of the duplicate cultures of each form. These estimated tube lengths were subtracted from the mean lengths of germ tubes in the cultures. This single subtraction corrected the measurements (1) for differences in the density of inoculum of different cultures, and (2) for the average difference between lengths of germ tubes of the two physiologic forms considered. After this subtraction, the results in duplicate cultures were averaged and thereafter considered together.

Up to this point the actual checks of the series were disregarded, largely to facilitate use of the correction for differences in density of inoculum. The checks could not be disregarded altogether since it was desired to put series run at different times on directly comparable bases, and the checks of individual series usually showed a tendency for series as a whole to diverge in one direction or the other from the averages used in calculating the estimated checks. Comparing the actual check measurements of series with estimated checks of the same densities of inoculum, definite figures were therefore obtained which represented the divergence of each check from the average. Averaging the individual divergences, a figure was secured which represented the mean divergence of the series for each form; and this was used to correct the results in the wheat extracts throughout the series. The "net increases in length of germ tubes" obtained after this second correction, listed in Table VI, appear as nearly perfectly comparable as pos-

sible with the information at hand. In the specific case of the two form-18 drop cultures in lot 6, Kanred extract, diluted to .01 in distilled water, the calculations involved were as given below:

	Culture a	Culture b	
Means of original measurements of lengths of germ tubes (there was unusually close agreement of duplicates here.)	518μ	513μ	
Number of urediniospores per drop	69	50	
Estimated check values, that is, estimated lengths of germ tubes in distilled water drops of corresponding densities of inoculum	181μ	165μ	
Recorded lengths minus estimated checks	337μ	348μ	
Average for the duplicate cultures			343μ
Divergences of measurements in actual check cultures of this series from estimated checks with the same density of inoculum as the actual checks			− 53μ + 92
Sum of divergences			+ 39
Mean divergence, for the series			+ 19μ

343μ − 19μ = 324μ, the net, corrected increase in length of germ tubes as used in Table VI.

The final corrected values indicate the increases only and are therefore smaller than the actual recorded lengths. Positive values indicate longer growth than in the checks and negative values the reverse.

It is evident from Table VI that the effect of extracts of either variety was usually to increase the length of germ tubes of both forms except in the most concentrated of the extracts of each series in which growth occurred. In Kanred extracts the increase was usually greater with form 18 than with form 19, but in Mindum extracts the greater increase was usually with form 19. This is demonstrated by the mean values for the different series, and for the experiment as a whole in Table VII, in which all the net increases from Table VI are averaged. Table VII includes also averages of results calculated without correction for the divergence of the actual checks from estimated check values. By both methods there was an average greater growth of form 18 in the Kanred series and of form 19 in the Mindum series. By both methods of calculation, this was true of the combined increases in lots 2, 4, 6, 7, and 8, which were not heated during preparation. With lots 3, 5, and 9, on the other hand, the increases in

the case of form 19 were greater than those of form 18 in the Kanred series as well as in the Mindum series.

The results given in Table VI were also considered by comparing the increase in each solution for the two forms. This was conveniently done by subtracting each form-19 "net increase" from the corresponding form-18 value. Positive differences indicate that in the particular substratum there were greater increases with germ tubes of form 18 than with those of form 19; negative differences indicate the reverse (Table VIII). Therefore, both the positive values shown for most of the Kanred extracts and the negative values for most of the Mindum extracts agree with the hypothesis that length of germ tubes is increased to a greater extent in extracts from susceptible plants than in those from resistant plants.

TABLE VIII

Differences Between Net Increases in Lengths of Germ Tubes of Forms 18 and 19*

Lot No.	Wheat extracts	Differences between increases for forms 18 and 19, in microns, in extracts diluted to concentrations indicated					
		Mean, all concentrations	1.0	.25	.1	.01	.001
2	Mindum	—278	....	....	—336	—226	—126
3	Kanred	—66	....	....	—61	—114	—22
	Mindum	31	....	—161	271	—66	78
4	Kanred	41	....	—108	97	299	—125
	Mindum	—130	....	—104	—153	—134	....
5	Kanred	85	—41	381	29	0	56
	Mindum	6	121	109	—20	—92	80
6	Kanred	140	....	49	14	131	365
	Mindum	—53	....	....	111	70	—341
7	Kanred	49	....	—34	—80	—6	317
	Mindum	—48	....	....	5	—176	26
8	Kanred	—22	....	....	....	—39	—5
	Mindum	26	....	....	....	144	—92
9	Kanred	—40	....	10	—44	—19	—108
	Mindum	—107	....	—98	—77	—40	—213

* Net increases, as given in Table VI, for form 19 were subtracted from the corresponding values for form 18.

Further condensation of results was obtained by subtracting the Mindum values from the corresponding Kanred values of Table VIII. These final subtractions yielded single figures, as given in Table IX, for each dilution of each lot, representing the summation of the differentiation between form 18 and form 19 in both the Kanred and the Mindum extracts prepared by the various methods. The series of subtractions involved in this summation of differentiation is expressed in the following algebraic equation, in which all the original values are of the net increases in lengths of germ tubes, as given in Table VI:

$$\text{summation} = (\text{form } 18_{\text{Kanred}} - \text{form } 19_{\text{Kanred}}) - (\text{form } 18_{\text{Mindum}} - \text{form } 19_{\text{Mindum}}).$$

Positive values obtained by this method indicate that elongation was increased to a greater extent in the extracts from susceptible varieties, and negative values that it was increased to a greater extent in extracts from the resistant varieties. In Table IX positive numbers predominate. The largest numbers, indicating most decided preferential elongation in susceptible rather than resistant extracts, are in the lots in which extracts were prepared without heating, that is, lots 4, 6, 7, and 8.

TABLE IX

SUMMATION OF LENGTH OF GERM-TUBE RESULTS OF EXPERIMENT 1, WITH VALUES CORRECTED, AS FOR TABLES VI AND VIII, FOR VARIATIONS IN DENSITY OF INOCULUM AND ALSO FOR DIVERGENCES OF SERIES FROM ESTIMATED MEANS*

Lot No.	Summation of length of germ tube results, in microns, in extracts diluted to concentrations indicated					
	Mean, all concentrations	1.0	.25	.1	.01	.001
3	—160	....	....	(—332)	—48	—100
4	226	....	(—4)	250	433	....
5	45	(—162)	272	49	92	—24
6	223	....	....	(—97)	61	706
7	125	....	....	(—85)	170	291
8	—48	....	....	....	(—183)	87
9	67	....	(108)	33	21	105

* Differences between net increases in lengths of germ tubes of forms 18 and 19, as given in Table VIII, for Mindum substrata were subtracted from the corresponding values for Kanred substrata. Positive numbers resulting from this final subtraction indicate that elongation was increased to a greater extent in extracts from susceptible varieties, negative numbers that it was increased to a greater extent in extracts from resistant varieties.

More decisive, and perhaps more accurate, comparison of lots is obtained when the last figures to the left in each row of the results in the individual dilutions (the figures in parentheses in Tables IX-XII) are omitted. It will be noted that the values are quite generally at variance with the others of the series. This is owing perhaps in part to the direct effect of the more concentrated extracts in inhibiting growth to such an extent as to obscure differences due to the varietal sources of the extracts, and in part to the fact that these numbers were of necessity based on fewer measurements than in the case of growth in more dilute substrata, since germination as well as growth was reduced in the higher concentrations. Considering, then, only those numbers to the right of the numbers in parentheses, it is to be noted that all the negative ones are included in the series in which the extracts were heated.

Tables X, XI, and XII were calculated to show that the results above were not based on errors due to the method of calculation. These tables are final summations of the results of subtractions as used in

TABLE X

LENGTH OF GERM TUBE RESULTS OF EXPERIMENT 1. SUMMATION AS IN TABLE IX, EXCEPT WITH VALUES CORRECTED FOR INDIVIDUAL VARIATIONS IN DENSITY OF INOCULUM BY THE USE OF ESTIMATED CHECKS, BUT NOT CORRECTED FOR SERIES DIVERGENCES

Lot No.	Summation of length of germ tube results, in microns, in extracts diluted to concentrations indicated					
	Mean, all concentrations	1.0	.25	.1	.01	.001
3	−62	....	....	(−234)	51	−2
4	227	....	(−3)	251	433	....
5	−48	(−254)	179	−48	−2	−119
6	171	....	....	(−99)	19	592
7	227	....	....	(46)	278	358
8	137	....	....	....	(7)	268
9	−41	....	(63)	−97	−106	−24

TABLE XI

LENGTH OF GERM TUBE RESULTS OF EXPERIMENT 1. SUMMATION AS IN TABLE IX, EXCEPT WITH VALUES CORRECTED BY THE ACTUAL CHECK CULTURES OF EACH SERIES AND NOT CORRECTED FOR VARIATION IN DENSITY OF INOCULUM

Lot No.	Summation of length of germ tube results, in microns, in extracts diluted to concentrations indicated					
	Mean, all concentrations	1.0	.25	.1	.01	.001
3	−14	....	....	(−235)	116	78
4	227	....	(22)	222	438	....
5	−5	(−70)	205	−26	−53	−80
6	176	....	....	(−17)	−17	562
7	30	....	....	(−97)	32	158
8	−80	....	....	....	(−228)	68
9	143	....	(307)	92	70	104

TABLE XII

LENGTH OF GERM TUBE RESULTS OF EXPERIMENT 1. SUMMATION AS IN TABLE IX, EXCEPT WITH ORIGINAL, UNCORRECTED VALUES*

Lot No.	Summation of length of germ tube results, in microns, in extracts diluted to concentrations indicated					
	Mean, all concentrations	1.0	.25	.1	.01	.001
3	−82	....	....	(−304)	48	10
4	233	....	(22)	222	455	....
5	47	(−20)	266	20	9	−36
6	261	....	....	(100)	118	558
7	152	....	....	(24)	153	278
8	203	....	....	....	(46)	360
9	32	....	(156)	36	32	−49

* The values tabulated here from the uncorrected measurements are the same as would be obtained by still another method of calculation, the use of an averaged check value for all form 18 cultures and a similar mean check for all form 19 cultures, as such average check corrections cancel out in the repeated subtraction used in the summation.

preparing Table IX, but differ in that the original values used in these subtractions were calculated again, independently, by the various methods indicated. Thus, Table X is based on the original measurements corrected only for variations in density of inoculum and for the average difference between forms 18 and 19, without using the actual checks of the series at all, thus obviating the possibility of bias

from them; Table XI is based on measurements corrected only with the actual checks of the series; and Table XII is based on original, uncorrected values.

Surprisingly close agreement was obtained by these various methods of calculating the results. Throughout the four tables, only one negative value was obtained for any of the concentrations to the right of the parentheses in lots 4, 6, 7, and 8. In the lots that were heated during preparation, on the contrary, there were 17 negative values. Averages of the results for the two series of extracts are given in Table XIII and demonstrate again the validity of the conclusion above. Irrespective of the method of calculation used, results in the unheated series are definitely in agreement with the hypothesis that elongation is increased to a greater extent in extracts from susceptible varieties. The lowest value obtained for the average increase was 104μ, and the highest, 314μ. It is to be remembered, however, that this is the sum of the average increase or decrease of both forms in both the Kanred and Mindum extracts.

TABLE XIII

MEANS OF TABULATED SUMMATIONS OF THE EFFECT OF KANRED AND MINDUM EXTRACTS IN DIFFERENTIATING FORMS 18 AND 19 BY THE INCREASES IN LENGTHS OF GERM TUBES*

Corrections applied to individual values	Means of summations, in microns			
	Lots 4, 6, 7, and 8 (unheated in preparation)		Lots 3, 5, and 9 (heated in preparation)	
	(a)†	(b)†	(a)	(b)
For variations in density of inoculum and by actual check values (Table IX)	148	285	1	44
For variations in density of inoculum only (Table X)	195	314	49	—18
By actual check values only (Table XI)	104	209	42	56
Uncorrected (Table XII)	213	306	—0.5	19

* Positive numbers indicate that elongation was increased to a greater extent in extracts from susceptible varieties, negative numbers that it was increased to a greater extent in extracts from resistant varieties.

† (a) Including all cultures of the series.

(b) Excluding results in the highest concentration of the substrata in each series in which comparison was possible.

With the series that were heated during preparation no such consistent differentiation was secured. The results for the different lots varied with the methods of calculation, and the averages in Table XIII show that in these series there was only negligible, if any, tendency for increase in elongation to be associated with the resistance or susceptibility of the varieties to the physiologic forms.

Branching ratios.—Germ tubes of form 19 characteristically branch more than those of form 18 on most substrata. This was true

in the present experiment, both in dilutions of the plant extracts and in sterile distilled water. Branching ratios—total lengths of branches divided by lengths of main germ tubes—for corresponding cultures in the same medium were often 10 to 20 times larger for form 19 than for form 18, with occasional ratios as large as .23 and .36 for form 19 in solutions in which the promycelia of form 18 did not branch at all. Table XIV lists these results as net increases or decreases, obtained by subtracting the ratios for the check cultures of the individual series from the respective ratios in extracts. The values here are therefore directly comparable between forms and presumably between series.

TABLE XIV

NET INCREASES IN BRANCHING RATIOS, *P. graminis tritici* FORMS 18 AND 19, IN EXTRACTS FROM KANRED AND MINDUM WHEAT, DILUTED IN STERILE, DISTILLED WATER*

Lot No.	Wheat extracts	Physiologic forms of *P. graminis tritici*	Net increases in branching ratios, in extracts diluted to concentrations indicated					
			Mean, all concentrations	1.0	.25	.1	.01	.001
2	Mindum	18	.087	.....	.....	0	.16	.10
		19	.172	.....	—.10	—.07	.23	.63
3	Kanred	18	.085	.....	.31	.14	—.06	—.05
		19	.287	.....	.....	.29	.22	.35
	Mindum	18	—.044	—.12	.07	—.06	0	—.11
		19	—.180	.....	—.04	—.27	—.14	—.27
4	Kanred	18	.007	.....	—.01	.01	—.01	.04
		19	.065	.....	0	—.09	.20	.15
	Mindum	18	.017	—.02	—.02	.07	.04	.....
		19	.030	.....	.01	.11	—.04	.04
5	Kanred	18	.042	—.05	—.01	.07	0	.20
		19	.120	—.03	.25	.07	.15	.16
	Mindum	18	.009	—.10	—.055	.31	—.10	—.01
		19	—.021	—.17	—.085	—.07	.02	.20
6	Kanred	18	—.041	.....	—.065	—.045	—.02	—.035
		19	—.036	.....	—.115	—.005	—.03	.005
	Mindum	18	.011	.....	.....	.057	—.003	—.02
		19	.015	.....	.....	—.13	.13	.045
7	Kanred	18	—.065	.....	—.05	—.08	—.05	—.08
		19	.039	.....	—.045	.035	.045	.12
	Mindum	18	.027	.....	.04	0	.06	.01
		19	.070	.....	.....	.20	—.01	.02
8	Kanred	18	0	.....	.....	.....	—.01	.01
		19	.017	.....	.....	.01	—.02	.06
	Mindum	18	0	.....	—.02	.....	—.01	.03
		19	—.057	.....	.....	—.02	—.10	—.05
9	Kanred	18	.060	—.05	—.01	.35	.05	—.04
		19	.290	.....	.26	.31	.60	—.01
	Mindum	18	.082	—.01	—.07	.32	.12	.05
		19	.225	.....	—.18	.97	.06	.05

* Branching ratios = total lengths of branches divided by lengths of main germ tubes. Increases figured from branching ratios in distilled water checks of the individual series.

As there is only very slight correlation of branching ratios with the number of spores per drop, corrections for variations in density of inoculum were unnecessary.

In all the 7 Kanred series, the means of increases in branching ratios for form 18 were lower than for form 19. In 4 of the corresponding Mindum series, also, the increases for form 18 were smaller than those for form 19, but in each of these cases the difference was less than in the respective Kanred series. There is a tendency for branching ratios to be greater in extracts from the resistant varieties than in extracts from susceptible varieties.

This relation is more readily evident in Table XV, in which are listed the differences between corresponding branching-ratio increases for forms 18 and 19. Positive values indicate that branching of form 18 increased more than that of form 19, negative values the reverse. In the Kanred series there are accordingly 18 negative values and only 7 positive values, while in the Mindum series there are 17 positive to the 10 negative values. Moreover, comparing the Kanred with the Mindum values, it will be noted that the latter are almost always larger. This is true with the means for the series in all except lot 6, where the difference is negligible.

TABLE XV

DIFFERENCES BETWEEN NET INCREASES IN BRANCHING RATIOS OF FORMS 18 AND 19*

Lot No.	Wheat extracts	Differences between increases in branching ratios, for forms 18 and 19, in extracts diluted to concentrations indicated						Average differentiation: Kanred mean minus Mindum mean
		Mean, all concentrations	1.0	.25	.1	.01	.001	
2	Mindum	—.210	.....	.....	.07	—.17	—.53	.....
3	Kanred	—.277	.....	.....	—.15	—.28	—.40	—.432†
	Mindum	.155	.....	.11	.21	.14	.16	
4	Kanred	—.057	.....	—.01	.10	—.21	—.11	—.060‡
	Mindum	.003	.....	—.03	—.04	.08	.....	
5	Kanred	—.078	—.02	—.26	0	—.15	.04	—.108†
	Mindum	.030	.07	.03	.38	—.12	—.21	
6	Kanred	—.002	.....	.050	—.040	.01	—.03	.002‡
	Mindum	—.004	.....	.....	.187	—.133	—.065	
7	Kanred	—.092	.....	.040	—.115	—.095	—.20	—.045‡
	Mindum	—.047	.....	.....	—.20	.07	—.01	
8	Kanred	—.020	.....	.....	.....	.01	—.05	—.105‡
	Mindum	.085	.....	.....	.....	.09	.08	
9	Kanred	—.205	.....	—.27	.04	—.55	—.04	—.360†
	Mindum	.155	.....	.11	.21	.14	.16	

* Net increases, as in Table XIV, for form 19 subtracted from corresponding values for form 18.

† Extracts heated during preparation.

‡ Extracts not heated during preparation.

Comparison of results with regard to the heating of extracts during preparation is of interest. When the means of values for the Mindum cultures are subtracted from those for the Kanred cultures, values are obtained which represent the average of all differentiation for each lot, as indicated in the last column of Table XV. There was better differentiation of forms to which the varieties are susceptible or resistant in the extracts that had been heated during preparation (lots 3, 5, and 9) than in the others (lots 4, 6, 7, and 8)—an effect exactly opposite to that noted in the differentiation on the criterion of increase in length of germ tubes.

Summary of Experiment 1.—In hanging-drop cultures with extracts from normal Kanred wheat plants, germ tubes of form 18 were increased in length, as compared with check cultures, to a greater extent than was the case with germ tubes of form 19 as compared to checks of form 19. In Mindum extracts, elongation of form 19 was correspondingly favored more than that of form 18. The average length of germ tubes increased more, in each case, in the extract from the susceptible variety. This relation appeared in the original measurements and still held when results were corrected for differences in density of inoculum, for differences between series, etc. The branching ratios, on the other hand, were increased to a greater extent in extracts from resistant varieties.

Experiment 2

Another series of plant extracts was prepared from Kanred, Mindum, and Little Club wheat seedlings, planted in the greenhouse May 21 and cut June 6, 1927. Approximately the same method of extraction was used as that for lot 4 of Experiment 1 (Table V). Plants were cut, washed with distilled water, ground, steeped for 3 hours in a quantity of distilled water equal to 2½ times the green weight of the plants, the liquid squeezed by hand through cheesecloth and ultrafiltered. Since some of the distilled water used in washing the plants always remained, the final filtrates were assumed to contain about .25 concentration of actual plant extract, and further dilution was made on this basis. Each extract was diluted in plain one per cent agar to final concentrations of .1, .01, and .001 for drop cultures. Three physiologic forms of *P. graminis tritici* were used as inoculum. The actual reactions of plants of the three varieties to these forms is shown in the following scale, running from "0" for immunity to "4" for extreme susceptibility:

Physiologic form	Little Club	Kanred	Mindum
1	4	0	1
18	4	4=	1=
19	4	0;	4=

The lengths of germ tubes produced in drop cultures of the extracts are given in Table XVI. These measurements have been corrected for variations in density of inoculum (using the averaged regression value derived from study of a number of forms of *P. graminis tritici* on agar drops, as explained above, Table II). They are thus directly comparable, each representing the residual length of germ tubes after all increase from density of inoculum has been removed; or in other words, representing the residual "constant" at the base, or at "o" number of spores per drop, of a number-of-spores to mean-length-of-germ-tube graph for each form on each substratum.

TABLE XVI

LENGTHS OF GERM TUBES OF PHYSIOLOGIC FORMS 1, 18, AND 19 OF *Puccinia graminis tritici* IN EXTRACTS FROM THREE WHEAT VARIETIES, DILUTED IN PLAIN ONE PER CENT AGAR*

Wheat extracts	Physiologic forms of *P. graminis tritici*	Lengths of germ tubes, in microns			
		In extracts diluted to concentrations indicated			In plain agar checks
		.1	.01	.001	
Little Club	1	264	165	211	249
	18	351	405	457	247
	19	245	373	235	332
Kanred	1	99	127	125	134
	18	344	198	179	331
	19	152	230	269	255
Mindum	1	234	445	400	408
	18	323	460	542	606
	19	211	406	427	358

* Corrected for variations in density of inoculum.

The values given in Table XVII have been further corrected for differences between the series by subtracting from each measurement for a diluted extract the check value for the particular series.

TABLE XVII

INCREASES IN LENGTHS OF GERM TUBES OF PHYSIOLOGIC FORMS 1, 18, AND 19 IN EXTRACTS FROM THREE WHEAT VARIETIES DILUTED IN ONE PER CENT AGAR, AS COMPARED TO LENGTHS IN CHECKS*

Wheat extracts	Physiologic forms of *P. graminis tritici*	Increases in lengths of germ tubes, in microns, in extracts diluted to concentrations indicated			
		Mean, all concentrations	.1	.01	.001
Little Club	1	−30	15	−84	−38
	18	157	104	158	210
	19	−48	−87	41	−97
Kanred	1	−17	−35	7	−9
	18	−94	13	−133	−152
	19	−58	103	−25	−46
Mindum	1	−18	−174	37	−8
	18	−174	−283	−146	−64
	19	−19	−147	48	69

* Corrected values, from Table XVI, for mean lengths of germ tubes in extracts, minus the respective lengths in check cultures.

In this experiment, the extracts generally inhibited growth as compared to that in the checks. Of the 27 sets of cultures in extracts, growth was less than that of the checks in 18, as shown by minus numbers in Table XVII. There was increased growth in only 9 cases. Of these, 8 were in the 15 sets with forms in extracts from varieties susceptible to those forms; while only one case, form 1 in .01 concentration of Mindum extract, was in the 12 sets of cultures in extracts from resistant plants. The average of growth minus that of respective checks for all cultures in susceptible extracts was about -5μ, or, even omitting the form 18 series in Little Club extracts, in which there were exceptional increases, only -46μ; while for cultures in resistant extracts the average was -74μ. It may be mentioned that this relation appeared also when the original data were tabulated without correction for differences in density of inoculum. The average in this case for all cultures in susceptible extracts was -27μ, and for cultures in resistant extracts, -67μ.

The relative branching of germ tubes in this experiment was studied in considerable detail; but these results appeared of little significance. The greatest decreases in branching ratios in the extracts as compared to those in the checks occurred in extracts from varieties susceptible to the respective forms, but so also did the greatest increases. The average of increases for all forms in all dilutions of varieties susceptible to them was .009, and for all forms in extracts from resistant plants, .012. This difference, tho slight, was in agreement with the results of Experiment 1.

Differentiation between forms to which varieties are resistant or susceptible was definite in this experiment only by the criterion of length of germ tubes. As in Experiment 1, the germ tubes were longer in extracts from susceptible varieties than in those from resistant varieties.

Experiment 3

The Mindum extract from Experiment 2 was used here with a larger number of physiologic forms. Lengths of germ tubes for 10 forms of *P. graminis tritici* are given in Table XVIII. In the 10 per cent concentration used, the Mindum extract decreased the growth of most of the forms to a point below that of the respective checks in plain agar.

The forms tested, however, grouped definitely into two series, with the differences in length running from $+58\mu$ to -93μ in one group and from -165μ to -203μ in the other. Of the seven forms to which Mindum is susceptible, there were five in the group of forms whose germ tubes were only slightly decreased in size; while of the three forms to which Mindum is resistant, there were two with the

TABLE XVIII

LENGTHS OF GERM TUBES, CORRECTED FOR VARIATIONS IN DENSITY OF INOCULUM, OF SOME PHYSIOLOGIC FORMS OF *P. graminis tritici* IN PLAIN-AGAR DROPS AND IN AGAR CONTAINING 10 PER CENT OF AN EXTRACT FROM MINDUM WHEAT

Physiologic forms of *P. graminis tritici*	Lengths of germ tubes, in microns		Increase or decrease in lengths of germ tubes, in microns, (length in Mindum agar minus that in checks) of forms to which Mindum is	
	10 per cent Mindum agar	Plain-agar checks	Susceptible	Resistant
1	131	319	....	−188
9	136	225	−89	....
15	211	414	−203	....
17	171	251	−80	....
18	197	362	....	−165
19	230	301	−71	....
21	183	276	−93	....
27	152	94	....	+58
34	61	118	−57	....
39	79	251	−172	....

more considerable decreases. The results then confirm in general the validity of the assumption that changes in cultural characteristics of forms in extracts from different varieties are to be attributed to the resistance relations of the varieties to the forms rather than to accidental variation.

The three discrepancies in Table XVIII are of particular interest. In correlation of the host ranges of a number of physiologic forms with the production of apical swellings by the same forms in drop cultures in plain agar (Fig. 11), all except four physiologic forms gave a comparatively close fit to a linear correlation. The four exceptions included three of the four forms studied to which Vernal emmer is susceptible (forms 9, 15, and 27) and form 39. In the present series, the three discrepancies include two of three forms studied to which Vernal emmer is susceptible (forms 15 and 27) and form 39.

Branching ratios for the series are listed in Table XIX. As before, there was general differentiation between the forms to which Mindum is susceptible and those to which it is resistant. The branching ratios were lowered with most of the forms in the first group, and raised with two of the three forms in the second group. There were three definite discrepancies. These again included form 39 and two of the forms to which Vernal emmer is susceptible, this time forms 9 and 27.

Differentiation by the percentages of teliospore-like bodies produced (Table XX) was not so uniform as with the other two criteria. It is probable that with most forms the production of apical bodies is increased to a greater extent in extracts from resistant varieties than in extracts from susceptible varieties. In the present series, coincident with the use of a substratum containing a high enough concentration of Mindum extract to decrease the length of germ tubes even of forms

TABLE XIX

RATIOS, TOTAL LENGTHS OF BRANCHES OVER LENGTHS OF MAIN GERM TUBES, OF SOME PHYSIOLOGIC FORMS OF *P. graminis tritici* IN PLAIN AGAR DROPS AND IN AGAR CONTAINING TEN PER CENT OF AN EXTRACT FROM MINDUM WHEAT

Physiologic forms of *P. graminis tritici*	Branching ratios		Increase or decrease (ratios in Mindum agar minus those of checks) of forms to which Mindum is	
	10 per cent Mindum agar	Plain-agar checks	Susceptible	Resistant
1	.005	.004	.....	+.001
9	.084	.013	+.071	.....
15	.023	.071	−.048	.....
17	.061	.080	−.019	.....
18	.008	.005	.....	+.003
19	.048	.089	−.041	.....
21	.025	.037	−.012	.....
27	.012	.035	.....	−.023
34	.062	.078	−.016	.....
39	.065	.....	+.065	.....

to which Mindum is susceptible, there was increased production of apical bodies with most of the forms as compared to that in the checks. The greatest increase was with form 1, to which Mindum is resistant, and the apical bodies here also resembled teliospores unusually closely. The only definite decreases were with forms 21 and 39, to which Mindum is susceptible. The greatest discrepancies in the results were found again in the case of physiologic forms to which Vernal emmer is susceptible, forms 15 and 27.

TABLE XX

PERCENTAGE OF GERM TUBE TIPS ON WHICH THE TELIOSPORE-LIKE APICAL SWELLINGS WERE PRODUCED WITH PHYSIOLOGIC FORMS OF *P. graminis tritici* IN PLAIN AGAR DROPS AND IN AGAR CONTAINING TEN PER CENT OF MINDUM EXTRACT

Physiologic forms of *P. graminis tritici*	Production of apical bodies, in per cent		Percentage in Mindum agar minus that in checks; for forms to which Mindum is			
	10 per cent Mindum agar	Plain-agar checks	Susceptible		Resistant	
			Uncorrected	Corrected*	Uncorrected	Corrected*
1	72	18	....	....	+54	+49
9	85	70	+15	+22	....	....
15	57	17	+40	+36	....	....
17	45	25	+20	+19	....	....
18	14	9	....	....	+5	+5
19	5	6	+5	+18	....	....
21	13	27	−12	−17	....	....
27	19	8	....	....	+11	−6
34	24	5	+19	+12	....	....
39	7	43	−36	−12	....	....

* Counts for individual cultures corrected for density of inoculum to 50 spores per drop.

Considering this experiment as a whole, two outstanding relations appear. First, most of the forms, 6 of 10, were divided, as a result of comparison of the growth in drop cultures containing 10 per cent of Mindum extracts to that in plain agar checks, into groups which

correspond definitely to the resistance of Mindum plants to the forms. Second, the greatest, and usually the only, discrepancies by all three criteria were in a definite group which included form 39 and those forms to which Vernal emmer is susceptible, and was identical with the group of forms found earlier to be aberrant to the usual correlation between rate of production of apical bodies and host-variety range.

Normal and Diseased Host Tissues as Sources of Extracts

Among theories on the nature of varietal resistance to rusts have been those postulating antibody action following infection. Two general hypotheses have been considered. First, antibodies produced under the stimulus of the parasite may inhibit the growth of rusts to which the plant is resistant. This inhibition may be supposed to act by direct attack on the parasite, in a manner more or less analogous to antibody resistance in animals; or by some indirect method such as increasing the sensitivity of adjacent host cells to the action of the parasite, with earlier death of these cells and eventual loss of nutrition for the pathogen. The second line of reasoning assumes that "antibody" formation, occurring perhaps only in the presence of physiologic forms of the rust to which the host is susceptible, produces in living host cells materials or conditions indispensable to the nutrition of the parasite. These materials might differ according to the plants and support the growth of only those rusts to which the hosts are susceptible. This second hypothesis would explain also the difficulty of growing rusts in artificial culture.

If either type of reaction actually is involved in resistance to rusts, extracts from plants attacked by rusts might include "antibodies"; and it is possible that such extracts might differentiate physiologic forms to which the host is resistant or susceptible more definitely than in the case of extracts from normal plants.

Material.—Kanred and Mindum wheat seedlings, grown in pots in the greenhouse during January to March, 1927, were used. Separate lots were used of the normal plants of each variety and of plants inoculated respectively with forms 18 and 19 of *P. graminis tritici* and grown in the greenhouse at the same time. Those inoculated with the different forms were separated from each other on the greenhouse bench only by glass partitions. Since it was desired that growth of the plants and also of the rusts with which the plants were inoculated should proceed under identical conditions for the three sets of each variety, and also that extracts should be tested as substrata for rusts within a few days of extraction, it was necessary to use seedlings of different ages for the different lots. Ages of the plants are given in Table XXI.

TABLE XXI

NORMAL AND DISEASED KANRED AND MINDUM WHEAT SEEDLINGS USED AS MATERIAL FOR EXTRACTS

Source of extract	Inoculated with *P. graminis tritici*	Age of plants when inoculated	Age of plants when cut	Infection record
		Days	Days	
Kanred	.....	..	14	.....
	Form 18	17	36	Abundant infection and sporulation
	Form 19	17	38	Hypersensitive flecking
Mindum	.....	..	9	.....
	Form 18	11	36	Hypersensitive flecking and necrosis
	Form 19	11	30	Abundant infection and sporulation

Method of extraction.—Plants were cut, weighed, placed in a clean funnel, and washed with a stream of distilled water to remove adherent spores and dust. They were then run through a meat grinder and mixed with an amount of distilled water three times the original green weight. The combined mass was pressed out between metal cups, using direct hand pressure only. The densely turbid, deep green fluid obtained was divided into two portions. The first was ultrafiltered through a Berkefeld filter, yielding a clear, straw-colored liquid. The second portion was passed through a single filter paper, using a previously heat-sterilized apparatus which prevented outside contamination. Usually the somewhat turbid, green filtrate still contained bacteria. The filtrates were stored in Pyrex tubes in the refrigerator and generally used within two days.

Cultures.—The filtrates themselves represented concentrations of approximately .25 that of the plant tissue; and further dilutions were made with sterile, distilled water to final concentrations of .1, .01, .001, and .0001. Hanging-drop cultures of forms 18 and 19 were made in duplicate with these dilutions of each filtrate.

Results.—Average estimated increases in lengths of germ tubes for each form on each substratum are given in Table XXII. As in previous series, germ tubes of form 18 increased in length to a greater extent in Kanred extracts than those of form 19, while in Mindum extracts, those of form 19 increased in length to the greater extent. In 26 sets of cultures of form 18 in the Kanred substrata, the germ tubes averaged 93 μ longer than those in estimated check cultures; while with form 19 the increases in the 25 pairs averaged only 32 μ. Again, in the Mindum extracts, the average with form 18 for 27 duplicated cultures was 12 μ, and 32 μ for 25 sets with form 19. These results agree with the susceptibility of Kanred to form 18 and its resistance to form 19, and the resistance of Mindum to form 18 and its susceptibility to form 19.

TABLE XXII

INCREASES IN LENGTHS OF GERM TUBES OF FORMS 18 AND 19 OF *P. graminis tritici* IN EXTRACTS, FROM NORMAL AND RUSTED WHEAT PLANTS, DILUTED IN STERILE, DISTILLED WATER*

Material	Filter	Physiologic forms of *P. graminis tritici*	Increases in lengths of germ tubes, in microns, in extracts diluted to concentrations indicated					
			Mean, all concentrations	.25	.1	.01	.001	.0001
Normal Kanred plants	Berkefeld	18	—76	....	—146	—121	....	39
		19	—102	....	—118	—169	....	—19
	Filter paper	18	—51	....	—130	—24	—61	12
		19	—77	....	—111	—164	—58	24
	Centrifuged, then Berkefeld	18	35	....	—49	—2	90	101
		19	17	....	—5	—2	—10	85
Kanred infected with form 18	Berkefeld	18	11	....	—46	124	36	—68
		19	31	—67	....	157	126	—90
	Filter paper	18	357	....	....	271	433	368
		19	171	....	—48	199	239	295
Kanred infected with form 19	Berkefeld	18	127	....	80	297	82	48
		19	—12	....	32	....	—26	—43
	Filter paper	18	272	....	246	287	329	225
		19	187	....	....	363	80	119
Normal Mindum plants	Berkefeld	18	—12	—138	—152	—44	133	140
		19	—30	....	—80	92	—60	—72
	Filter paper	18	—17	....	—176	—118	133	92
		19	10	....	—133	39	128	5
Mindum infected with form 18	Berkefeld	18	11	....	—38	—12	112	—17
		19	1	....	61	—70	—55	68
	Filter paper	18	—27	—14	—112	—53	0	46
		19	94	....	2	237	32	104
Mindum infected with form 19	Berkefeld	18	—25	—155	—102	98	56	—20
		19	4	—34	—3	—34	31	58
	Filter paper	18	163	....	220	293	206	—65
		19	114	....	—89	201	179	165

* Recorded lengths minus (distilled water) checks estimated for the same densities of inoculum.

Comparing the growth obtained in the different extracts given in Table XXII, it is to be noted that longer germ tubes were usually produced in the extracts filtered through filter paper than in corresponding extracts passed through the Berkefeld filter. Furthermore, differentiation between the length increases of forms 18 and 19 in the same extracts was almost always greater in the portions passed through filter paper only (Table XXIII). It is then perhaps permissible to consider the results in the paper-filtered series and to disregard the somewhat erratic results of the Berkefeld-filtered series.

It is evident as shown in Table XXII that longer germ tubes were produced in the extracts from the infected plants than in those from the normal plants. On the basis of these figures it would also appear that the longest growth occurs on extracts from plants inoculated with

TABLE XXIII

DIFFERENCES BETWEEN INCREASES IN LENGTHS OF GERM TUBES FOR FORMS 18 AND 19, *P. graminis tritici*, IN EXTRACTS FROM NORMAL AND RUSTED WHEAT PLANTS*

Material		Filter used	Differences between increases in length of germ tubes, in microns, in extracts diluted to concentrations indicated				
			Mean, all concentrations	.25	.1	.01	.001	.0001
Kanred plants	Normal	Berkefeld	—26		—28	48		58
		Filter paper	—27		—19	140	—3	—12
		Centrifuged, then Berkefeld	18		—44	0	100	16
	Infected with form 18	Berkefeld	—34		—33		—90	22
		Filter paper	113		72	194		73
	Infected with form 19	Berkefeld	50		—48		108	91
		Filter paper	93		—76	249		106
Mindum plants	Normal	Berkefeld	49		—72	—136	193	212
		Filter paper	—27		—43	—157	5	87
	Infected with form 18	Berkefeld	10		—99	58	167	—85
		Filter paper	—123		—114	—290	—32	—58
	Infected with form 19	Berkefeld	—28	—121	—99	132	25	178
		Filter paper	49		309	92	27	—230

* Mean increases in lengths of germ tubes for form 18, corrected for variations in density of inoculum, minus similarly corrected means for form 19.

forms to which they are susceptible. Thus, in the Kanred series the average for form 18 in the extract from normal plants is —51μ; in the extract from plants infected with form 19, 272μ; and in that from plants successfully attacked by form 18, 357μ. Similarly, both physiologic forms of *P. graminis tritici* produced longer germ tubes in the Mindum extracts from plants inoculated with form 19 than in the extracts from those inoculated but not infected with form 18, to which Mindum is resistant. Differences between the results obtained with extracts of plants inoculated with form 18 and those secured with extracts of plants inoculated with form 19 are not necessarily significant, since actual check cultures of these series were disregarded by the method of calculation of Table XXII; but the very large differences between growth in extracts of inoculated plants and growth in extracts of normal plants can hardly be considered accidental.

More accurate comparison of different series is possible with the differences between increases with form 18 and 19 than with the values themselves. In Table XXIII, the increases for form 19 in each substratum are subtracted from the corresponding values for form 18. Positive values indicate greater increases, or smaller decreases, for form 18, and negative values greater increases for form 19. As

would be expected, the majority of such subtractions in the Kanred series gave positive values and the majority in the Mindum series negative values.

Averages for the differentiation in extracts prepared from the various materials by filtration through filter paper were as follows:

Kanred (form 18—form 19),	
Normal	27μ
Infected with form 18 (susceptible)	113
Infected with form 19 (resistant)	93
Mindum (form 18—form 19),	
Normal	27
Infected with form 18 (resistant)	123
Infected with form 19 (susceptible)	49

In both series, differentiation was definitely higher in the extracts from plants infected with form 18 than in the extracts from the normal plants. It may be noted that this relation, as well as the other results above, was still more definite when the results were calculated without using the corrections for variations in density of inoculum.

Branching-ratio results in the extracts from normal and diseased material are given in Table XXIV. As already noted, germ tubes of form 19 usually branch much more than those of form 18. This was evident again in the present experiment. In only one group of cultures of the 48 in which comparison is possible was there a higher branching ratio for form 18 than for form 19; while the form 19 ratio was higher in 42 cases.

It was also found above (Tables XIV and XV) that branching ratios were higher in the extracts from normal plants of resistant varieties than in extracts from susceptible varieties. In the present series, this was true except with the cultures of form 18 in extracts filtered through filter paper.

The branching ratios were influenced to a marked extent by the method of preparing the extracts. In every series, the averages for form 19 ratios (Table XXIV) were greater in the Berkefeld-filtered substrata than in those filtered merely through filter paper; while with form 18 the reverse was true in four of the six series. Differentiation of the two forms by the characteristic differences between the branching ratios was thus emphasized in the ultrafiltered extracts of most series.

Differences between branching ratios, with respect to the normal and diseased sources of substrata, were rather erratic. Branching-ratio values for differences between the two physiologic forms did not always vary inversely with the differences in lengths of germ tubes, as would have been expected from the other results. The relations

TABLE XXIV

BRANCHING RATIOS FOR FORMS 18 AND 19 OF *P. graminis tritici* IN EXTRACTS, FROM NORMAL AND RUSTED WHEAT PLANTS, DILUTED IN STERILE, DISTILLED WATER*

Material	Filter used	Physiologic forms of *P. graminis tritici*	Branching ratios in extracts diluted to concentrations indicated					
			Mean, all concentrations	.25	.1	.01	.001	.0001
Normal Kanred plants	Berkefeld	18	.008	.....	0	0	.....	.025
		19	.160	.....	.105	.182	.....	.192
	Filter paper	18	.031	.....	0	.027	.053	.042
		19	.154	.....	0	.250	.270	.096
	Centrifuged, then Berkefeld	18	0	.....	0	0	0	0
		19	.237	.....	0	.221	.296	.431
Kanred infected with form 18	Berkefeld	18	0	.....	0	0	0	0
		19	.246	.111	.....	.121	.286	.467
	Filter paper	18	.016	.....	.....	.006	.017	.025
		19	.059	.....	0	.063	.085	.086
Kanred infected with form 19	Berkefeld	18	.009	.....	0	.014	0	.022
		19	.110	.....	.037	.....	.077	.215
	Filter paper	18	.005	.....	0	.007	.002	.012
		19	.095	.....	.....	.013	.111	.161
Normal Mindum plants	Berkefeld	18	.012	0	0	0	.043	.018
		19	.108	.....	0	0	.352	.080
	Filter paper	18	.027	.....	0	0	.010	.097
		19	.042	.....	0	.038	0	.128
Mindum infected with form 18	Berkefeld	18	.007	.....	0	0	0	.026
		19	.154	.....	.128	.158	.127	.204
	Filter paper	18	.020	0	0	.041	.045	.016
		19	.140	.....	.069	.197	.170	.123
Mindum infected with form 19	Berkefeld	18	.045	0	.114	.026	.066	.019
		19	.203	.040	.269	.160	.453	.094
	Filter paper	18	.035	.....	.005	.021	.012	.100
		19	.107	.....	.006	.035	.192	.196

* Total lengths of branches divided by lengths of main germ tubes.

here are complex and certainly involve more than merely the development of branches instead of main germ tubes in "uncongenial" substrata.

Summary.—The growth from urediniospores of *P. graminis tritici* forms 18 and 19 was studied in hanging-drop cultures in extracts from normal Kanred and Mindum wheat plants, and from plants inoculated with the two rusts. Differentiation of growth of the forms, to which the varieties are reciprocally resistant and susceptible, in the extracts from normal plants confirmed the results of earlier experiments. The length of germ tube was greater in extracts from the diseased plants than in those from normal plants, and differences between the two forms also were intensified in the extracts from infected plants. Branching-ratio differentiation was not demonstrated so clearly in the extracts from diseased plants.

Some Characteristics of Differentiating Materials in Extracts

Stability in stored extracts:

Forms 18 and 19 in stored extracts.—Part of the extracts of lot 4 of Tables V to XV was stored in the refrigerator for eight months, in Pyrex tubes plugged with cotton, and then used in the preparation of hanging-drop cultures of forms 18 and 19. The stored material was diluted to one per cent in three different materials: plain one per cent agar; one per cent agar containing as nutrient 10 per cent of Shive's solution (20); and sterile, distilled water. The mean lengths of germ tubes as given in Table XXV have been corrected for variations in density of inoculum only. It is to be noted that the uncorrected figures showed precisely the same relations as those given here.

TABLE XXV

DIFFERENTIATION OF FORMS 18 AND 19 OF *P. graminis tritici* IN KANRED AND MINDUM EXTRACTS PREVIOUSLY STORED IN THE REFRIGERATOR FOR EIGHT MONTHS

	Physiologic forms of *P. graminis tritici*	Extracts diluted to .01 in: Plain 1% agar		1% agar + 10% Shive's solution		Distilled water	
		Kanred	Mindum	Kanred	Mindum	Kanred	Mindum
Mean lengths of germ tubes, corrected for density of inoculum, in microns	18	481	304	316	218	367	299
	19	203	242	215	158	211	198
	18–19	278	62	101	60	156	101
Ratio, lengths of branches divided by lengths of germ tubes	18	.006	.002	.002	.007	.007	.003
	19	.031	.060	.009	.067	.015	.160
Production of apical swellings*	18	Trace	+	Trace	+	?	+
	19	?	Trace	+	—	Trace	?

* + = apical swellings present; ? = doubtful; — = absent.

As usual, forms 18 and 19 were clearly different on every substratum. Germ tubes of form 18 were longer in each case than those of form 19, but those of the latter branched more.

Comparing growth in Kanred substrata to that in the Mindum series, unusually consistent differentiation of forms to which the varieties are susceptible or resistant was obtained. In each of the three diluents form 18 germ tubes exceeded those of form 19 in length to a greater extent in the Kanred than in the Mindum cultures. Except for form 19 in the plain agar series, there was a higher production of the apical, teliospore-like bodies on germ tubes of each form in extracts from the resistant than from the susceptible plants. Contrary to results in previous series, however, branching ratios were greater in extracts from the susceptible variety than from the resistant one.

Refrigeration for eight months did not destroy the property in these extracts which differentiates forms to which the varieties are resistant or susceptible. In the present series, differentiation is clear using any one of three different materials with which to dilute the extracts, except that differentiation by branching ratios appears to have been reversed.

Forms 1 and 17 in stored extracts.—Such excellent differentiation was secured with forms 18 and 19 in the previous series that the same stored Kanred and Mindum extracts were used for cultures of forms 1 and 17. Kanred is immune and Mindum is moderately resistant to form 1, while Kanred is immune also from form 17 and Mindum is very susceptible to it. Stakman and Levine (30) symbolize these relations as follows:

	Kanred		Mindum	
Form	Range	Mean	Range	Mean
1	0 to 0;	0	0 to 2	1
17	0 to 1=	0	3− to 4++	4=

Data regarding the growth of these forms in the Kanred and Mindum extracts diluted to 0.01 in plain one per cent agar, and in plain agar checks, are given in Table XXVI. Lengths of germ tubes here were corrected for variations in density of inoculum as in Table XXV. Since form 1 produces the apical, teliospore-like bodies abundantly, the actual percentages of tips of germ tubes that produced these bodies were counted in the present series.

TABLE XXVI

DIFFERENTIATION OF FORMS 1 AND 17 OF *P. graminis tritici* IN KANRED AND MINDUM EXTRACTS PREVIOUSLY STORED IN THE REFRIGERATOR FOR EIGHT MONTHS

	Physiologic forms of *P. graminis tritici*	Substrata		
		0.01 Kanred in plain agar	0.01 Mindum in plain agar	Plain agar checks
Mean lengths of germ tubes (corrected for variations in density of inoculum), in microns	1	79	141	258
	17	148	276	217
Ratio, length of branches over length of germ tubes	1	.010	.040	.042
	17	.158	.087	.106
Percentage of germ tubes producing apical swellings	1	86	27	38
	17	10	18	0

As would be expected, form 1 germ tubes were smaller in both of the much diluted extracts than in the checks. Moreover, germ tubes were shorter in the extract from the immune Kanred than in the extract from the very resistant Mindum. Also according to hypothesis, form 17 germ tubes were longer in the Kanred extract

than those in the checks and smaller in the Mindum cultures than in the checks.

The results were as inconsistent as those of the preceding series with respect to the extent of branching and the production of apical swellings. Only the branching ratios with form 17, and the production of apical swellings with form 1, were as expected. As in Figure 7, D and B, some of the apical swellings produced by form 1 in the Kanred agar closely resembled teliospores, while those in the checks were fewer in number and of the more usual clavate shapes.

Considering both series, it is apparent that despite the lack of differentiation of all forms by all criteria, the characteristics of the four physiologic forms of *P. graminis tritici* when cultured on these extracts, eight months after extraction, were sufficiently different to indicate the actual variations in resistance of the wheat varieties to the four forms.

Stability to heating.—In Experiment 1 extracts made by processes involving cooking of any sort were of less value in differentiating forms by length of germ tubes than were extracts prepared without heating. From this it appeared possible that the materials which differentiate physiologic forms are thermolabile, or, if not, are affected by other results of the heating. However, in cultures in which extracts were diluted in plain agar, the extracts were held at 44.5° to 45.5° C., both when diluted and undiluted, for intervals often approaching two hours, apparently without lowering the differential value of the extracts. As a preliminary test, the experiment summarized in Table XXVII was performed. Cultures were prepared of Mindum extract diluted to 10 per cent in plain one per cent agar: this agar was then heated at

TABLE XXVII

DIFFERENTIATION OF PHYSIOLOGIC FORMS OF *P. graminis tritici* IN 10 PER CENT MINDUM AGAR, IN CULTURES PREPARED IMMEDIATELY AFTER DILUTION AT 45° C., AND OTHERS PREPARED AFTER HEATING AGAR AT 97° C. FOR 15 MINUTES

	Physiologic forms of *P. graminis tritici*	Growth in			Growth in Mindum agar minus that in checks	
		10% Mindum agar		Plain agar checks		
		Heated	Unheated		Heated	Unheated
Mean lengths of germ tubes (corrected for variations in density of inoculum), in microns	1	47	132	319	−272	−187
	18	30	197	363	−333	−166
	19	172	230	301	−129	−71
Ratio, length of branches over length of main germ tubes	1	0	.005	.004	−.004	+.001
	18	0	.008	.005	−.005	+.003
	19	.067	.048	.089	−.022	−.041
Percentage of germ tubes producing apical swellings	1	15	72	18	−3	+54
	18	9	15	0	+9	+15
	19	0	5	0	0	+5

97° C. for 15 minutes, cooled rapidly to 45° again, and a second series of cultures inoculated.

Mindum is resistant to forms 1 and 18, and susceptible to form 19. The results in the unheated agar are therefore in agreement with those of the previous experiments. Compared with the checks, mean lengths of germ tubes of forms 1 and 18 were much less than those of form 19. Branching ratios were slightly larger for forms 1 and 18 in the Mindum agar and considerably smaller for form 19 than in the corresponding checks. The production of apical swellings was increased markedly with forms 1 and 18. The only disagreement was in the slight increase in production of apical swellings with form 19 in the Mindum agar as compared to the check.

In the heated Mindum agar, growth was in general inhibited to a marked extent. Lengths of germ tubes were much shorter than in either of the other media; however, the average length of germ tubes of form 19 was still considerably more than that for either of the other two forms, and differentiation of the forms by the branching ratios was reduced but not lost.

It may tentatively be considered that those constituents in wheat extracts which can differentiate physiologic forms to which the variety is susceptible or resistant are probably not entirely destroyed by heating at 97° C. However, this heating reduces the value of the extract as a substratum for the rusts, and hence the differentiation may well be obscured in some heated extracts. From the results in Experiment 1, it seems possible that autoclaving may result in complete disappearance from extracts of the materials concerned in differentiation by lengths of germ tubes, with perhaps accentuation of the differentiation by the branching ratios.

Filtration methods as affecting differentiation.—In the experiment with extracts from normal and rusted Kanred and Mindum plants, a portion of each extract was filtered through a single filter paper and another portion ultrafiltered through a Berkefeld filter. The summarized results as to lengths of germ tubes for forms 18 and 19 in these extracts are given in Table XXIII. Correct differentiation here is indicated by positive values in the Kanred series and negative values in the Mindum series. It will be noted that there was greater differentiation in the extract filtered only through filter paper in every series except the Mindum infected with form 19. Averaging all values in the table, the relation is marked:

	Kanred series	Mindum series
ultrafiltered	14μ	7μ
filtered through paper	77μ	—67μ

Differentiation by length of germ tubes was undoubtedly more pronounced in the rather turbid green filtrate which passed through filter paper than in the clear ultrafiltrate in this series. The differentiating materials may be located in the chloroplasts, which would agree with the conception that growth of rusts is intimately connected with photosynthesis of the host. It would be equally plausible, however, to consider that differentiation in elongation may be due to completely soluble materials, present equally in both filtrates; but that differentiation may be magnified in the presence of additional purely nutritive and non-differentiating materials in the chloroplasts, or otherwise contained in greater concentration in filtrates through filter paper.

In the same ultrafiltered and paper-filtered extracts, the differences in branching ratios were intensified in the ultrafiltrates, since form 19 ratios were larger in them and the form 18 ratios usually smaller. Differentiation by branching ratios is possibly the result of materials or conditions in the extracts other than those concerned in the differentiation by lengths of germ tubes.

Tests of the Production of Toxins by Rust Sporelings

Varietal resistance to physiologic forms of rusts is probably due to either, or conceivably both, of the following general relations: (1) variations in the nutritive value of the host to the parasite; and (2) variations in the actual resistance of host varieties to the toxic activities of the different physiologic forms. The former hypothesis is the one on which most of the experiments in the present paper have been planned, but some preliminary tests of the latter hypothesis also are given.

The second hypothesis may be considered to involve, essentially, the ability of susceptible host tissues to tolerate such toxic materials as may be excreted by the parasite or produced by the host under stimulation from the parasite. All other tissues, resistant or immune, will not tolerate or withstand these hypothetic toxic materials, so that host cells in the vicinity of the point of infection die; the typical "hypersensitive area" is produced; and the parasite, thus cut off from its living nutrient substratum, soon dies also.

If this hypothesis is correct, it might be possible to demonstrate its probability by exposing various host tissues to the action of liquid media in which rust spores have grown. Resistant plants, if actually more susceptible to the toxic substances, should suffer greater injury than susceptible tissues. Direct determination by somewhat similar methods of the production by plant pathogens of materials toxic to the host tissues has been successful with various species of *Fusarium*, following the work of Haskell (11) and of Falmy (9) with *Fusarium*

solani. So far as the writer knows, there is no previous record of this type of experiment with rusts.

Hypodermic Injection of Culture Filtrates

Mass sowings of urediniospores of *P. graminis tritici* forms 14 and 27 were made in an extract, from normal Kanred wheat plants, which was diluted in sterile, distilled water to .01 concentration and used 5 cc. per culture in Erlenmeyer flasks. Three flasks were used for each form. The cultures were incubated at 23° C. for three days and the liquid was then filtered through sterile filter paper, using aseptic precautions, to remove the thin surface films of spores and germ tubes. Composite samples of the filtrates for each form were injected into the stems of 17-day-old wheat seedlings. Ten plants each of Marquis, Mindum, Speltz Marz, and Vernal emmer were used for each of the culture composites and for the check filtrate from the uninoculated blanks.

Injections were by means of finely drawn glass tubing with tips of approximately .2 mm. outside diameter and .15 to .16 mm. inside diameter. As much liquid as possible was forced into the stems, leaving always a drop of the liquid standing over the puncture as the needle was withdrawn. Only a slight amount of liquid appeared to be retained in the punctures. Each plant was injected at two or three points between the first and second nodes above ground. The plants were kept in the greenhouse as before injection.

Observations were made for three weeks. Six days after the injections definite but very narrow brownish necrotic halos, probably due to the mechanical injury, appeared around all of the punctures. No further injury developed, nor were there any differences between varieties or between the culture and check-filtrate injections in the extent of the injury.

Absorption of Culture Filtrates

The negative results of the previous experiment might be attributed to the fact that too little of the liquid was introduced to cause injury. In further tests, portions of the same culture filtrates were used again, but in such a way as to introduce larger quantities into the plants.

Wheat seedlings grown 18 days in the greenhouse were cut off at the surface of the ground, rinsed immediately in distilled water, and the stems cut back again while still in the water. The plants were placed immediately in individual small glass tubes, of 4 mm. outside diameter, 6.5 cm. long, and sealed at the bottom. The tubes, previously filled nearly to the tops with filtrates, or with sterile, distilled water in the case of checks, were then supported in cardboard racks on the greenhouse bench. As the various liquids were taken up by the plants,

sterile, distilled water was added from time to time to keep the tubes almost full. Thus, irrespective of the rate of imbibition, each plant eventually absorbed approximately the same amount of the original solution as did the others.

The results were decisively negative. Plants treated as described lived for three days to a week in different series. The form 14 filtrate was tried with Marquis, Khapli, Little Club, and Vernal emmer wheat seedlings; and the form 27 filtrate with Marquis and Khapli seedlings. In each case both check filtrates from uninoculated blanks and distilled water were used for comparison. The type of liquid appeared to have not the slightest influence on the prolongation of greenness or turgidity or on the prevention of wilting. All plants of the same variety showed the same gradual decline and death, differing in time for the different varieties, regardless of their differing substrata.

The results indicate either that the fungus does not produce materials toxic to wheat tissues, or that the methods used were not adequate to demonstrate such materials. In any case, no toxic activity by the fungus was shown. This agrees with the results previously discussed, which indicate that physiologic resistance can be explained by differences, in the normal plant constituents, which affect the growth of the various physiologic forms of *P. gramminis tritici.*

DISCUSSION

Considerable differences were found in the growth of uredinial germ tubes of different physiologic forms in hanging-drop cultures of water, plain agar, and other substrata (Fig. 1). These differences were consistent, and indicate that physiologic forms of *P. graminis tritici* may be differentiated, in hanging-drop cultures under specified conditions, by the type of growth, the length of germ tubes, and the relative abundance of branches on the promycelium, in much the same way as physiologic forms of other fungi (6, 8) have been identified by cultural characteristics. It must be noted, however, that the present work has not shown whether the differences found between forms of *P. graminis tritici* are true of all isolations of a given form, or are characteristic merely of those studied. More than one isolation of a given physiologic form was rarely studied in this investigation. In preliminary comparison of two isolations of form 39, there was rather close agreement of characteristics in drop culture; but many more isolations must be studied before it can be concluded that the characteristics observed in drop cultures always correspond with pathogenic characteristics.

The foregoing statement does not apply to the relation between the rate of production of apical swellings and the host range of physiologic

forms of *P. graminis tritici*. There seems to be a positive correlation between the readiness with which these teliospore-like bodies are produced and the narrowness of host ranges of the forms.

It appears unlikely that the production of apical swellings bears a direct causal relation to the restriction of host range with which it appears to be associated. We may, however, consider it tentatively as an indication of those physiologic potentialities of the fungus which are concerned in resistance. Thus, if mycelium from germinating urediniospores tends to enter almost immediately a reproductive stage which inhibits further vegetative growth, and this tendency varies in intensity in different physiologic forms, then for a particular form the percentage of germ tubes that produce apical swellings under specified conditions may serve as an index of the intensity of this tendency toward reproduction. To complete this hypothesis, we may assume further that physiologic forms that produce many apical bodies, and in which the "reproductive" tendency is accordingly high, the host range is relatively restricted because the conditions required for successful vegetative growth are more highly specific than those necessary for the growth of physiologic forms that produce fewer apical swellings.

A converse relation may also be deduced from this correlation. If the production of apical swellings is an index to extensiveness of host ranges, it would appear from the correlation that the twelve varieties selected by Stakman and Levine as representative of wheat varieties were correctly chosen.

It is in accord with our general idea of the complex nature of physiologic resistance that it should be true that the correlation between the percentages of germ tubes that produce apical swellings and the extent of the host ranges of the forms is not applicable to the entire population of physiologic forms studied. The correlation applies to the majority of physiologic forms, but apparently does not hold for form 39 and several forms to which Vernal emmer is susceptible. These same forms were again found to be the only ones aberrant in other respects, as reported elsewhere in this paper. In studying the growth of physiologic forms in extracts from susceptible and resistant plants, it was found that differences in cultural characteristics of all forms studied corresponded with differences in pathogenicity except with form 39 and those forms to which Vernal emmer is susceptible. Were these discrepancies not confined to a definite group, they might constitute a serious objection to the general idea that the drop-culture characteristics studied in this paper are to be considered evidence of the physiologic interrelations of host and parasite which may be concerned in physiologic resistance to rusts. As it is, from the consistent

way in which these same forms were aberrant to the various types of results, the writer would instead consider the following explanation more probable.

A series of relations concerned in resistance to most physiologic forms of *P. graminis tritici,* but perhaps not extending to the Vernal-emmer group, which may for convenience be considered to include form 39 also, may be demonstrated in artificial culture by the growth of the physiologic forms in extracts. The Vernal-emmer group is possibly included in this group so far as physiologic resistance in the plant is concerned; but demonstration of the relation in drop-culture growth is masked perhaps by differences in environmental characteristics. It would be equally plausible, however, to consider that some factors involved in resistance to the Vernal-emmer group are important only with that group, and are of little or no importance in resistance to the other physiologic forms that are affected primarily by the relations demonstrable in culture. The writer has no explanation to offer for the fact that form 39 so persistently and clearly resembles the Vernal-emmer series, altho this variety is so resistant to form 39. It is evident that the basis for the reactions in artificial culture of the forms constituting this group – distinguished, it is to be remembered, merely by aberrancy—is not the same as that which enables a form to parasitize Vernal emmer successfully.

The most significant results of this work appear to be those bearing on the nature of resistance of wheat varieties to *P. graminis tritici.* Physiologic resistance to stem rust appears to be based at least in part on the composition of the normal host cells, entirely in the absence of any infection. Despite the slight mycelial growth obtained from urediniospores in hanging-drop cultures of even susceptible varieties, there were constant differences between the growth of the physiologic forms which agreed with the susceptibility of the varieties to the forms. In extracts from plants susceptible to a given form, germ tubes were in general longer than in check cultures; while in extracts from resistant plants, the germ tubes were shorter than in the checks. Germ tubes branched more and produced a higher percentage of apical swellings in extracts from resistant plants than in those from susceptible plants, altho these differences did not occur regularly with the aberrant physiologic forms of the Vernal emmer group.

These differences in germ tube growth in the various extracts, diagnostic of the resistance relation of the forms to the varieties, appeared consistently in extracts prepared from normal plants. In one experiment the differentiation was intensified in extracts prepared from infected seedlings. This may indicate some antibody formation, altho extraction from infected or dead cells of inoculated plants may be more

thoro and merely furnish a higher yield of materials concerned in resistance, which are also present in the normal tissues.

The results indicate that physiologic resistance is caused by the inability of the fungus to survive in tissues of resistant plants, an inability based on the composition of the normal tissues. Whether it is due to the presence in susceptible tissues of specifically nutritive materials which are absent in resistant tissues, or to the presence in resistant tissues of toxic materials absent in the susceptible tissues, cannot be determined with the information at hand. Either aspect of the hypothesis assumes that injury to the tissues of resistant host plants comes from the toxic materials given off by the dying or dead fungus cells in the uncongenial resistant tissues. It may be noted that in other cases in which the basis of physiologic resistance has been explained as due to differences in the composition of the normal host plants Walker (32) has considered that resistance to onion smudge is due to the presence of specific toxic substances in the scales of resistant onions, and Reynolds (24) has suggested that resistance to *Fusarium lini* is based on greater concentrations of glucosides in the resistant flax plants.

Whether the materials concerned in physiologic resistance, and serving also to differentiate physiologic forms in extracts from the plants, are primarily in the resistant plants, the susceptible ones, or both, it is clear that they are effective in considerable dilution. Approximately the same relations between physiologic forms were shown in the present work in dilutions of extracts to .001 or .0001 as in the most concentrated solutions in which successful growth could be secured. Extracts stored in the refrigerator for eight months were still capable of differentiating forms to which the varieties are resistant or susceptible, whence it would appear that the materials concerned are relatively stable. In Experiment 1, extracts prepared by heating did not differentiate physiologic forms so clearly by the differences in lengths of germ tubes as extracts prepared without heating; yet differentiation was still possible in series of cultures prepared (Table XXVII) with extracts which, after being mixed with the agar diluent, had been heated to 97° C. for 15 minutes. Differentiation by lengths of germ tubes was more definite in extracts filtered only through filter paper rather than through the Berkefeld filter; while differentiation by differences in the branching ratios was more definite in the ultrafiltrates. Apparently more than one series of materials is concerned in differentiation by these various cultural characteristics, unless one of the characteristics is affected by much more dilute concentrations of the host materials than is the other.

Further investigation of this sort, in which the living sporelings are

used as indicators of the results obtained by fractionations of plant extracts, should yield a more accurate idea of the materials actually involved in resistance to rusts and perhaps also of those utilized in their nutrition.

SUMMARY

1. The growth from urediniospores of *Puccinia graminis* was studied in hanging-drop cultures. Growth beyond about 1,000 μ was seldom obtained. Cultural characteristics, such as length of germ tubes, the type of growth, and abundance of branching, differed in these sporelings with different physiologic forms of *P. graminis tritici,* tho these characteristics are constant for given strains under controlled conditions. It has not yet been proved whether these cultural characteristics are true of all isolations of the physiologic forms, or are characteristic merely of particular strains within them.

2. There is high positive correlation between the number of urediniospores with which a drop is seeded and the final length of germ tube per spore. This has been worked out for many forms of rusts and for a number of substrata. The increase in length of germ tubes with increased density of inoculum is great enough so that corrections were calculated and applied, to insure that conclusions based on differences in lengths of germ tubes would not be influenced by the unavoidable differences in density of inoculum in the experiments.

3. Apical swellings on the tips of germ tubes, regarded as occasional or abnormal by earlier workers, were produced by all forms of all species studied to any considerable extent. These bodies resemble teliospores morphologically, are produced by different physiologic forms of *P. graminis tritici* in approximately the same relative abundance as normal teliospores on infected wheat plants, and are probably immature teliospores. The production of apical, teliospore-like bodies is negatively correlated with the number of spores per drop.

4. Teliospore-like bodies are produced by the physiologic forms of *P. graminis tritici* under identical conditions on a purely synthetic medium—plain one per cent agar—at rates relatively constant per form and widely varying between forms. The rates vary in linear correlation with the extensiveness of the host (variety) ranges of the forms, for almost the entire population of physiologic forms tested. Percentages of germ tubes producing teliospore-like bodies are higher in artificial cultures of forms with relatively limited ranges and progressively lower with physiologic forms of more extensive host ranges. A definite group of forms of *P. graminis tritici,* including form 39 and those of the forms tested to which Vernal emmer is susceptible, are aberrant to the linear correlation noted.

5. Extracts prepared from wheat varieties differed in their ability to support the growth of physiologic forms of *P. graminis tritici* in exact agreement with the respective resistance or susceptibility of the varieties to the various forms. Congeniality of forms to host varieties was evidenced almost universally by greater lengths of germ tubes in extracts from susceptible varieties, less branching, and lower production of apical swellings, than in extracts from resistant varieties. All forms of *P. graminis tritici* tested fit the groupings of resistance by their reactions in culture to host extracts, except those forms to which Vernal emmer is susceptible and form 39, the same group that was aberrant to the linear correlation. Evidently there are at least two series of relations involved in resistance to *P. graminis tritici*, unless these aberrant forms differ from the majority in some minor environmental responses that interfere with cultural results without affecting the relation between the parasites and their hosts.

6. In a single experiment, differentiation was greater with extracts from inoculated plants than in extracts from normal plants. Antibody activity may be, but is not necessarily, involved.

7. Extracts stored in the refrigerator for eight months still contained materials that differentiated between forms to which the variety concerned is susceptible or resistant. Extracts prepared by methods involving heating apparently did not contain the materials affecting germ tube elongation. A partial sterilization of extracts by filtration through a single, sterile filter paper instead of the usual filtration through a Berkefeld filter produced substrata of greater differentiating activity as to lengths of germ tubes, but of less differential value with regard to differences in the branching of germ tubes.

8. Materials extractible from normal as well as infected wheat tissues are able to affect the growth of physiologic forms of *P. graminis tritici*, in artificial culture in the extracts, in a manner diagnostic of the resistance of the host to the various physiologic forms. These materials in the plant tissues presumably explain the phenomenon of physiologic resistance to stem rust.

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